Markers of The Sleep-Wake History in the Human Electroencephalogram and their Relationship to Cortical Connectivity

Author(s):
Fattinger, Sara

Publication Date:
2017

Permanent Link:
https://doi.org/10.3929/ethz-b-000173371 →

DISS. ETH NO. 24077

MARKERS OF THE SLEEP-WAKE HISTORY IN THE HUMAN ELECTROENCEPHALOGRAM AND THEIR RELATIONSHIP TO CORTICAL CONNECTIVITY

A thesis submitted to attain the degree of
DOCTOR OF SCIENCES of ETH ZURICH
(Dr. sc. ETH Zurich)

presented by
SARA CORINNA FATTINGER
M.Sc. ETH Zurich

born on 23.06.1986
citizen of Arlesheim (BL)

accepted on the recommendation of
Prof. Dr. D. P. Wolfer, examiner
Prof. Dr. R. Huber, co-examiner
Prof. Dr. O. G. Jenni, co-examiner

2017

Table of Contents

Homeostasis

"The coordinated physiological processes which maintain most of the steady states in the organism are so complex and so peculiar to living beings…. that I have suggested a special designation for these states, homeostasis. The word does not imply something set and immobile, a stagnation. It means a condition - a condition which may vary, but which is relatively constant"

W.B. Cannon (1932)

1. Summary

Sleep is a tightly homeostatically regulated process across 24 hours. Sleep pressure increases with the time spent awake and dissipates with the progression of sleep (Borbély, 1982). These changes in sleep pressure have been related to changes in synaptic strength (Tononi and Cirelli, 2003). Synaptic strength is thought to increase during wakefulness due to learning processes favouring synaptic potentiation and to be renormalized during sleep when the brain is disconnected from the environment. High synaptic strength increases cortical excitability which is reflected in a better synchronized neuronal activity pattern (Esser et al., 2006). In the spontaneous electroencephalogram (EEG) neuronal synchrony is best reflected in the slope of sleep slow waves (0.5-2 Hz, Esser et al., 2007) the EEG hallmark of deep sleep (*i.e*. slow wave sleep). A recent study reported evidence that the level of neuronal synchronisation is also reflected in the waking EEG (Vyazovskiy et al., 2011). The authors observed a local intrusion of sleep-like brain activity (*i.e*. local sleep) in the EEG of awake rats under condition of high sleep pressure. Such local sleep on the neuronal level were detectable as theta waves (5-8 Hz) in the EEG. According to the synaptic homeostasis hypothesis (SHY) the underlying neuronal activity pattern of slow waves, is thought to reduce synaptic strength (Tononi and Cirelli, 2014). However, up to date there is no direct evidence for such a causal role of slow waves in the regulation of synaptic strength. Thus, the overall goal of the current thesis was to investigate the function of slow waves in the homeostatic regulation of synaptic strength in humans. To do so, we investigated 3 major aims with 6 research articles (RA).

Aim 1 elaborated on different electrophysiological markers of sleep homeostasis and network connectivity in the sleep and wake EEG during development (RA 1-3). Human brain development from early infancy to adulthood represents a time window in which massive changes in cortical connectivity and brain function occur: Synaptic density increases during the first years of life, peaks shortly before puberty, which is followed by a reduction during adolescence (Huttenlocher, 1978). Concurrently, white matter maturation progressively increases, improving the functional integration of distant brain regions (Fair et al., 2007; Paus et al., 2001). In the first RA we investigated the development of sleep homeostasis during the first year of life, by examining the sleep dependent dynamic of the slope of slow waves. We found an overnight decrease in the slope of slow waves in infants, indicating that in humans sleep homeostasis develops as early as the first year of life. At late childhood, synaptic density reaches its maximum, thus the drive of sleep pressure increase during the day might be very strong at this age (Carskadon et al., 2004; Jenni et al., 2005). Therefore, we examined whether theta activity in the wake EEG of children might fulfil signs of local intrusion of sleep-like activity during wakefulness (RA 2). Using waking high-density (hd) EEG recordings we found that theta waves show similar properties, as local sleep discovered on the cellular level in awake rats (Vyazovskiy et al., 2011). In the 3rd RA we studied the development of long-distant connectivity from late childhood to adulthood. We could show that approximate entropy, an information based measure, significantly increased with age indicating better long range connectivity (Pincus, 1994) in adults compared to teenagers. Thus, such increase in long-range connectivity with age might improve the integration of different brain networks, which may contribute to the age-related improvement of cognitive demanding tasks (Luna et al., 2004). In sum,

these results demonstrate that changes of cortical connectivity are reflected in the firing pattern of cortical neurons which can be detected on the surface by different electrophysiological markers.

The 2nd aim translated these findings into a clinical population. Childhood epilepsies with an activation of epileptic activity (*i.e*. spike waves) during slow wave sleep, are characterized by a severely disturbed slow wave sleep. Assuming that slow waves are causal for the sleep-dependent renormalization of synaptic strength, the question arises whether this restorative function of sleep can be fulfilled in these patients. To answer this question, we focused on the sleep-dependent decrease of the slope of slow waves which is thought to reflect the renormalization of synaptic strength. Taken together, we intended to demonstrate that spike wave activity during slow wave sleep impairs the physiological overnight reduction of the slope of slow waves (RA 4-5). Our results revealed that the sleep-dependent slope decrease was impaired in these patients, which might be due to the pathological epileptic activity. These findings provide some evidence for an active role of sleep slow waves in the regulation of synaptic strength. As these results are only correlative, a direct manipulation of either slow waves, or synaptic strength would be needed to prove causality.

Hence, aim 3 intended to selectively manipulate slow wave sleep to establish a causal function of slow waves on the homeostatic regulation of synaptic strength (RA 6). Recent findings have demonstrated that slow waves can be specifically manipulated by auditory stimulation, if tones were delivered time-locked to the ongoing slow waves (Ngo et al., 2013). Therefore, we developed a closed-loop auditory stimulation tool to detect slow waves in real time. This tool was then combined with hd-EEG measurements which allowed us to disturb slow waves selectively in one particular brain area, without interfering with the global structure of sleep. Thus, to achieve our last aim we assessed cortical excitability by transcranial magnetic stimulation in the evening and morning after one night of sleep where we applied our hd-EEG closed-loop auditory stimulation tool. We found that the sleep-dependent recovery of cortical excitability over left primary motor cortex was diminished, when slow waves were focally perturbed over this brain area. These results provide first direct evidence for a causal role of sleep slow waves on the regulation of synaptic strength in humans. In conclusion, our hd-EEG closed-loop auditory stimulation tool allows to selectively disturb slow waves over one particular brain area without interfering with the global structure of sleep. The value of such a tool is further increased by the recent finding of slow wave enhancement by auditory stimulation (Ngo et al., 2013). As such, this simple and non-invasive approach would be ideally suited for clinical application. For example, new therapeutic approaches during sleep could modulate specifically affected brain areas without the negative consequences of insufficient sleep on daytime performance.

2. Zusammenfassung

Schlaf ist ein homöostatischer, über 24 Stunden präzise regulierter Prozess: der Schlafdruck nimmt im Wachzustand zu und baut sich im Schlaf wieder ab – die sogenannte homöostatische Regulation des Schlafes (Borbély, 1982). Diese Veränderungen im Schlafdruck wurden mit der synaptischen Stärke, die im Wachzustand aufgrund von Lernprozessen zunimmt und im Schlaf wieder abgebaut wird, in Verbindung gebracht (Tononi und Cirelli, 2003). Eine zunehmende synaptische Stärke im Wachzustand erhöht die kortikale Erregbarkeit und wird durch ein sehr synchrones neuronales Aktivitätsmuster reflektiert (Esser et al., 2006). Die neuronale Synchronisierung wird im spontanen Elektroenzephalogramm (EEG) am besten in der Steigung der *Slow Waves* (0.5-2 Hz), den typischen EEG-Wellen des Tiefschlafes, widerspiegelt (so genannter *Slow-Wave-Schlaf,* Esser et al., 2007). Darüber hinaus konnte eine kürzlich veröffentlichte Studie zeigen, dass der Grad der neuronalen Synchronisierung auch im Wach-EEG reflektiert wird (Vyazovskiy et al., 2011). Dabei wurde in wachen, schlafdeprivierten Ratten beobachtet, dass Theta-Wellen (5-8 Hz) im Wach-EEG eine lokale, schlafähnliche neuronale Hirnaktivität (d.h. lokalen Schlaf) widerspiegeln. Die Synaptische-Homöostase-Hypothese (SHY) geht davon aus, dass die zugrunde liegende neuronale Aktivität der *Slow Waves* für die Reduktion der synaptischen Stärke im Schlaf verantwortlich ist (Tononi und Cirelli, 2014). Bis heute gibt es jedoch keinen direkten Beweis für eine aktive Beteiligung der *Slow Waves* an der Regulation der Synapsenstärke. Das Hauptziel dieser Arbeit war es daher, die Funktion der *Slow Waves* in der homöostatischen Regulation der Synapsenstärke beim Menschen zu untersuchen. Dazu wurden drei Hauptziele in sechs wissenschaftlichen Artikeln (WA) verfolgt.

Ziel eins bestand darin, verschiedene elektrophysiologische Marker der Schlafhomöostase und der neuronalen Netzwerkverbindungen im Schlaf- und Wach-EEG während der Entwicklung zu untersuchen (WA 1-3). Die Entwicklung des menschlichen Gehirns von der Geburt bis zum Erwachsenenalter stellt ein Zeitfenster mit enormen Veränderungen in kortikalen Verbindungen und Hirnfunktionen dar. Dabei nimmt die Dichte der Synapsen während der ersten Lebensjahre zu, erreicht das Maximum kurz vor der Pubertät, und nimmt während der Adoleszenz wieder ab (Huttenlocher, 1978). Gleichzeitig entwickelt sich die weisse Substanz kontinuierlich (Paus et al., 2001), wodurch die Funktionsintegration von weiter entfernten Hirnregionen verbessert wird (Fair et al., 2007). Im ersten WA wurde die Entwicklung der Schlafhomöostase während des ersten Lebensjahres anhand der schlafabhängigen Dynamik der Steigung der *Slow Waves* analysiert. Wir konnten zeigen, dass bei Säuglingen die Steigung über die Nacht abnimmt. Dies deutet auf eine frühe Entwicklung der Schlafhomöostase beim Menschen hin. In der späten Kindheit erreicht die Dichte der Synapsen ihr Maximum, was zu einem beschleunigten Aufbau des Schlafdrucks im Wachzustand beitragen könnte (Carskadon et al., 2004; Jenni et al., 2005). Im Rahmen des zweiten WA konnten wir mithilfe von hochauflösenden (hd) EEG-Aufzeichnungen zeigen, dass Theta-Wellen im Wach-EEG von Kindern die gleichen Eigenschaften aufweisen wie lokaler Schlaf auf zellulärer Ebene bei wachen Ratten (Vyazovskiy et al., 2011). In unserem dritten WA analysierten wir die Entwicklung von Verbindungen über längere kortikale Distanzen von der späten Kindheit bis zum Erwachsenenalter. Wir konnten zeigen, dass die sogenannte *Approximate Entropy*, ein Mass basierend auf der Informationsverarbeitung, mit steigendem Alter signifikant zunimmt. Dies

könnte eine Stärkung von längeren kortikalen Verbindungen bei Erwachsenen im Vergleich zu Jugendlichen reflektieren (Pincus, 1994). Infolge könnte die Funktionsintegration von weiter entfernten Hirnregionen verbessert und hierdurch wiederum die altersabhängige Verbesserung von schwierigen kognitiven Aufgaben gefördert werden (Luna et al., 2004). Zusammengefasst zeigen diese Resultate, dass sich Veränderungen in der kortikalen Netzwerkverbindung im Aktivitätsverhalten der kortikalen Neuronen widerspiegeln, welches mit Hilfe von verschiedenen elektrophysiologischen Markern auf der Oberfläche gemessen werden kann.

Im Rahmen des zweiten Zieles bauten wir auf diesen Resultaten auf, um die Funktion der *Slow Waves* in der Regulation der synaptischen Stärke in einer klinischen Population zu untersuchen. Kinder, die eine Epilepsie mit Aktivierung der epileptischen Potentiale (so genannte *Spike-Waves*) im Schlaf aufweisen, zeigen einen stark pathologisch veränderten *Slow-Wave-Schlaf*. Davon ausgehend, dass *Slow Waves* für die schlafabhängige Reduktion der synaptischen Stärke notwendig sind, stellt sich die Frage, ob diese Erholungsfunktion bei solchen Patienten noch erfüllt wird. Um dieser Frage nachzugehen, untersuchten wir bei diesen Patienten die schlafabhängige Reduktion der Steigung der *Slow Waves*, welche die Abnahme der synaptischen Stärke reflektiert (WA 4-5). Zusammengefasst zeigen unsere Resultate, dass die schlafabhängige Reduktion der Steigung bei diesen Kindern vermutlich aufgrund der *Spike-Wave* Aktivität gestört ist. Diese Resultate deuten darauf hin, dass *Slow Waves* eine aktive Rolle in der Regulation der synaptischen Stärke spielen. Dennoch besitzt dies nur korrelative Aussagekraft – für einen eindeutigen Beweis der Kausalität der *Slow Waves* wäre eine direkte Manipulation der *Slow Waves* oder der synaptischen Stärke notwendig.

Um einen kausalen Zusammenhang zwischen den *Slow Waves* und der homöostatischen Regulation der Synapsenstärke zu untersuchen, war es daher das dritte Ziel, den *Slow-Wave-Schlaf* spezifisch zu manipulieren (WA 6). Eine kürzlich erschienene Studie hat gezeigt, dass *Slow Waves* durch auditorische Stimulation spezifisch manipuliert werden können, wenn die Töne während einer bestimmten Phase der *Slow Waves* gespielt werden (Ngo et al., 2013). Wir entwickelten deshalb ein Gerät für auditorische Stimulation, welches uns erlaubt, *Slow Waves* in Echtzeit zu detektieren. Dieses System kombinierten wir mit hd-EEG Aufzeichnungen, um *Slow Waves* in einer bestimmten Hirnregion zu manipulieren, ohne jedoch die gesamte Schlafstruktur zu stören. Im Rahmen dieses letzten Ziels manipulierten wir in der Nacht *Slow Waves* lokal und massen die kortikale Erregbarkeit mittels transkranieller Magnetstimulation sowohl am Abend zuvor als auch am darauf folgenden Morgen. Wir konnten zeigen, dass die schlafabhängige Erholung der kortikalen Erregbarkeit im linken primär-motorischen Kortex gestört war, wenn die *Slow Waves* in dieser Hirnregion lokal manipuliert wurden. Dieses Resultat liefert erste direkte Hinweise auf eine kausale Funktion der *Slow Waves* in der Regulation der synaptischen Stärke. Zusammenfassend ermöglicht unsere Methode der auditorischen Stimulation eine lokale Manipulation der *Slow Waves*, ohne die globale Schlafstruktur zu stören. Kürzlich gelang es auf ähnliche Weise die *Slow Waves* zu erhöhen (Ngo et al., 2013). Somit bietet diese Methode eine einfache und nicht-invasive Möglichkeit für klinische Anwendungen. Unter anderem könnten daraus neue therapeutische Ansätze abgeleitet werden, um, ohne den Schlaf selbst und damit verbunden die Tagesleistung zu stören, im Schlaf mit der Aktivität spezifischer Hirnregionen zu interagieren.

3. Introduction

Sleep is a universal phenomenon that is ubiquitous across phylogeny (Campbell and Tobler, 1984) . On a behavioural level, it is defined as a "reversible state of perceptual disengagement from and unresponsiveness to the environment" (Carskadon and Dement, 2011). Typically, sleep is associated with postural recumbence, immobility and closed eyes. However, besides this rather simple behavioural definition, sleep is also considered a complex physiological process of the brain, driving the body to rest (Hirshkowitz, 2004). The regulation of the sleep-wake-cycle, *i.e.* the timing, duration and quality of both, wake and sleep, is determined by the interaction of two independent processes (Borbély, 1982): the circadian process C and the homeostatic process S. Process C relies on the function of the suprachiasmatic nucleus (SCN), which is located at the anterior hypothalamus and is considered to be the master circadian clock (Moore and Eichler, 1972; Rosenwasser and Turek, 2015). The SCN generates a self-sustained circadian oscillation driven by a transcriptional-translational loop (Jin et al., 1999; Reppert and Weaver, 2002), entraining internal rhythms to the daily light-dark cycle. Thereby, process C influences sleep distribution over 24-h periods (Franken and Dijk, 2009). The function of Process C in relation to sleep regulation as well as the underlying molecular mechanisms (*i.e.* expression of different clock genes in the SCN itself but also in peripheral cells) has been intensively studied and is well understood (Rosenwasser and Turek, 2015). The second process determining sleep-wake regulation is the homeostatic process S, relating sleep pressure to the sleep-wake history. Process S can also be described as an 'hour-glass': sleep need and the propensity to initiate sleep gradually increases with time spent awake and dissipates in the course of sleep (Achermann and Borbély, 2003). These changes in sleep pressure are best reflected in the sleep electroencephalogram (EEG) or local field potential (LFP) by sleep slow waves, the hallmark of deep sleep (sleep slow waves will be further described in the following paragraphs). It has been observed both in animals (Larkin et al., 2004; Trachsel et al., 1992) and humans (Dijk and Czeisler, 1995) that the homeostatic regulation of sleep does not depend on a functioning circadian system. This led to the established assumption that process S and C are two functionally and anatomically separated processes. Nevertheless, the interaction between the two processes enables sustained cognitive performance and alertness, even during prolonged wakefulness (Borbély, 1982): with increasing time awake, the endogenous circadian rhythm counteracts the accumulating homeostatic drive for sleep to maintain a stable level of neurobehavioral functioning. In contrast, process C facilitates consolidated sleep towards the morning, when sleep pressure has dissipated (Cajochen et al., 2002, 1999). Compared to process C, and despite a wealth of scientific investigations during the last decades, the fundamental function of process S remains elusive, which has triggered the proposal of different hypotheses in the field. Among others, there are two established hypothesis for the function of sleep homeostasis, which are mainly based on electrophysiological and neurobiological observations: the "synaptic homeostasis hypothesis" (also called "SHY") by Tononi and Cirelli (Tononi and Cirelli, 2014, 2006) and the hypothesis that sleep allows individual neurons to perform prophylactic cellular maintenance (here also called "sleep for the single neuron") by Vyazovskiy and Harris (Vyazovskiy and Harris, 2013). In the current thesis, I will comprehensively discuss both hypotheses. Based on their assumptions, the goal was to quantify different parameters of the homeostatic regulation of sleep in the surface EEG of humans. In a further

step, we developed a non-invasive tool, aiming to selectively manipulate the homeostatic process of sleep to investigate causality.

3.1 Vigilance states: Basic electrophysiological principles of wake and sleep

Using surface EEG recordings, the different vigilance states are defined based on characteristic brain activities (*e.g*. frequency and amplitude). In virtually all mammals and birds studied to date, three main vigilance states, *i.e.* wakefulness, rapid eye movement sleep (REMS) and non-REM sleep (NREMS) have been described. REMS and NREMS are as distinct from one another, as each is from wakefulness. The waking brain activity is characterized by fast frequencies and low amplitude waves. Under normal physiological conditions, the transition from wake to sleep is through NREMS (Carskadon and Dement, 2011). Falling asleep is a gradual process, following a progressive deepening of sleep, which is reflected in the different sub stages of NREM sleep (N1-N3): sleep transits from N1 to N3 as sleep deepens. The hallmark of deep sleep (*i.e*. N3) are sleep slow waves, high amplitude (> 75 µV) waves between 0.5 and 2 Hz. Therefore, N3 sleep is also termed slow wave sleep. NREMS is followed by REMS characterized by wake-like brain activity but accompanied by rapid eye-movements and muscle atonia. Such a sequence of NREMS and REMS is defined as one sleep cycle, which lasts approximately 80 to 100 minutes in humans and is repeated several times across a sleep episode (Carskadon and Dement, 2011; Iber et al., 2007).

As already mentioned above, slow waves during NREMS are the best physiological correlates of process S, *i.e*. the homeostatic regulation of sleep. Since this work focuses on the electrophysiological markers of process S in the wake and sleep EEG, in the following, the term *sleep* refers to NREMS.

3.1.1 Underlying cellular mechanism of sleep slow waves

In a first step, the electrophysiological mechanism underlying slow wave generation will be introduced. A schematic illustration of the underlying neuronal activity pattern of sleep slow waves is shown in Figure 1. During wakefulness, cortical neurons are tonically depolarized and fire irregularly giving rise to an EEG pattern displaying low amplitudes and fast activities. Upon falling asleep, the activity pattern changes. For short intermitted periods, cortical neurons cease their firing activity resulting in slower and higher amplitude waves in the EEG (Steriade et al., 2001). The deeper we fall asleep the more frequently these periods of silence occur. During profound slow wave sleep, virtually all cortical neurons engage in the so-called slow oscillation (<1 Hz), consisting of an active period (*i.e*. on-state) and a silent period (*i.e*. off-state). Combining multi-unit activity (MUA; extra cellular recording of several nearby neurons) and LFP recordings *in vivo*, Vyazovskiy and colleagues (2009) have demonstrated that these transitions between on- and off-states are highly synchronized between individual units and are therefore detected as high amplitude slow waves on the surface. In particular the termination of the active on-state is highly synchronized which produces the characteristic EEG pattern of sleep slow waves (Volgushev, 2006). Furthermore Vyazovskiy and colleagues (2009) revealed the temporal relationship between the slow oscillation on the cellular level and slow waves on the surface: the active on-state of the slow oscillation, corresponds to the up-phase and the silent off-state to the down-phase of sleep slow waves.

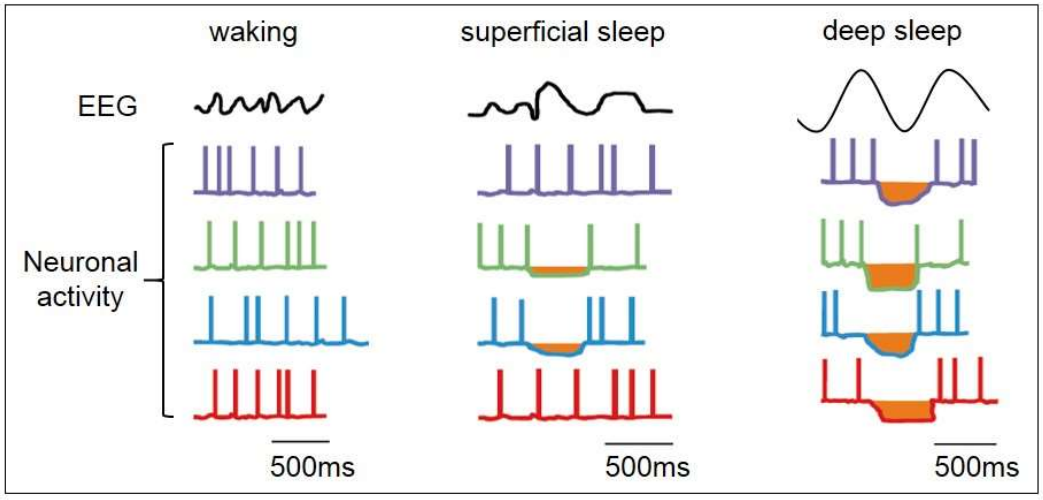

Figure 1

Schematic illustration of the underlying neuronal activity pattern of wakefulness, superficial sleep and deep slow wave sleep. Off-states on the neuronal level are indicated by a lowering of the membrane potential (orange area; adapted from Vyazovskiy & Harris 2013).

The origin and generation of this slow oscillation of cortical neurons during NREMS, first described by Steriade et al., (1993b), were investigated in great details on the cellular and molecular level (Achermann and Borbély, 1997; Steriade et al., 1993a, 1993b, 1993c; Timofeev et al., 2001). Decreased activity of arousal-promoting neuromodulators (*i.e.* acetylcholine and norepinephrine) during NREMS increases the conductance of the potassium leak channels, thereby reducing the resting membrane potential of cortical neurons (Hill and Tononi, 2005). Such drive towards hyperpolarization leads to a bi-stability of the resting membrane potential of cortical neurons, thereby triggering the slow oscillation. More specifically, the slow oscillation appears as an alternation of the membrane potential between two stable voltage levels: a depolarized on-state when neurons are active (firing frequencies similar to wakefulness) and a hyperpolarized off-state characterized by neuronal quiescence (no firing activity). There are three primary active intrinsic currents of cortical neurons which underlie the on- and off-state of the slow oscillation. During the hyperpolarized off-state, the persistent voltage-dependent sodium current ($I_{Na(p)}$), and the hyperpolarization-activated cation current (I_h) gradually increase, pushing the cell to a depolarized state. In addition, excitatory synaptic input from the thalamus may also terminate the off-state. On the other hand, during the depolarized on-state, the activity-dependent depolarization-activated potassium current (I_{DK}) initiates the next off-state. As I_{DK} is activity-dependent, the amount of depolarization and spiking activity during the on-state determines the degree of the following hyperpolarization (Hill and Tononi, 2005).

In the human EEG, important features of sleep slow waves are that they are locally regulated (Huber et al., 2004) and that they travel across the cortex (Massimini et al., 2004). Using high-density (hd) EEG recordings in humans, it has been demonstrated that slow waves originate mainly over frontal areas and propagate along an anteroposterior axis (Massimini et al., 2004). Later, multisite intracellular recordings

in cats confirmed this observation on the cellular level. Volgushev and colleagues demonstrated, that the active on-state of the slow oscillation predominantly originates in the anterior location and spreads preferentially along the anteroposterior direction (Volgushev, 2006). Another important hallmark of sleep slow waves is that they are homeostatically regulated. This will now be discussed in more details it the following section.

3.1.2 Homeostatic regulation of sleep: From single neurons to EEG activity

Slow waves can be quantified by slow wave activity (SWA), which corresponds to the EEG power between 0.5-4.5 Hz (Achermann 2009). SWA increases as a function of prior wakefulness and decreases in the course of sleep (Achermann and Borbély, 2003). Hence, increased sleep pressure after prolonged wakefulness (*i.e.* sleep deprivation or sleep restriction), enhances SWA (Borbely et al., 1981), whereas, napping during the day reduces SWA in the following night (Werth et al., 1996). Using MUA these changes in SWA are also detectable on the level of neuronal activity (Rodriguez et al., 2016; Vyazovskiy et al., 2009). These studies demonstrate that the sleep-wake history is reflected in the firing pattern of cortical neurons. At the beginning of a night when sleep pressure is high, the on- and off-states of the slow oscillation are highly synchronized between individual units, associated with high amplitude, steep slow waves (which, in turn leads to high SWA). When sleep pressure dissipates with the progression of sleep, this synchronization declines resulting in lower amplitude slow waves (which in turn leads to reduced SWA). Furthermore, these changes in the level of synchronized neuronal network activity are best reflected in the slope of slow waves. Highly synchronized transitions from the on- to the off-state within neuronal populations give rise to a steep slope of slow waves. Thus, in sum, the homeostatic regulation of sleep pressure (accumulation during wakefulness and its release during sleep) is not only reflected by SWA but can also be measured at the neuronal level (Rodriguez et al., 2016; Vyazovskiy et al., 2009).

During the last decades, evidence has mounted that the sleep-wake history is not only reflected in the sleep EEG but can also be detected during wakefulness. However, compared to SWA in the sleep EEG, theta activity (EEG power between 4.5-8 Hz) seems to reflect the homeostatic regulation of the sleep-wake cycle in the wake EEG. Theta activity parallels subjective sleepiness and serves as an objective marker of sleep pressure during prolonged wakefulness (Aeschbach et al., 1999; Akerstedt and Gillberg, 1990; Strijkstra et al., 2003). Accordingly, theta activity increases during sleep deprivation, with a similar dynamic to the wake-dependent increase of SWA during NREMS (Aeschbach et al., 1997a; Cajochen et al., 1995; Finelli et al., 2000; Hung et al., 2013). However, due to the interaction of Process C and S such an increase in theta activity becomes only detectable if the organism is challenged by prolonged waking hours (*i.e.* after sleep deprivation or sleep restriction). The reason is that the endogenous circadian rhythm counteracts accumulating sleep pressure during waking to maintain stable levels of neurobehavioral functioning under normal physiological conditions (Cajochen et al. 2002; Cajochen et al. 1999).

In short, as the time course of SWA across a sleep episode resembles the dissipation of sleep pressure with the time spent asleep, accumulating theta activity during prolonged wakefulness might reflect the

build-up of sleep pressure while awake. Also on the neuronal level, there is further evidence for a close relationship between theta activity during wakefulness and SWA during sleep. In a recent study, using MUA in awake, freely moving rats, Vyazovskiy and colleagues (2011) could detect intermitted brief off-states in a subset of cortical neurons, which were associated with theta activity in the LFP. Strikingly, the occurrence of such off-states in the primary motor cortex was related to reduced performance in a fine-skilled motor task. On the electrophysiological level, these off-periods were essentially indistinguishable from the off-states underlying sleep slow waves (for a representative example see Figure 2). However, compared to the global altering pattern of virtually all cortical neurons during slow wave sleep, the off-states during wakefulness occurred locally and lasted only a few ms. Nonetheless, with increasing sleep pressure during sleep deprivation, these off-states became more frequent and more global, involving different subsets of cortical neurons spread over the cortex (Vyazovskiy et al., 2011).

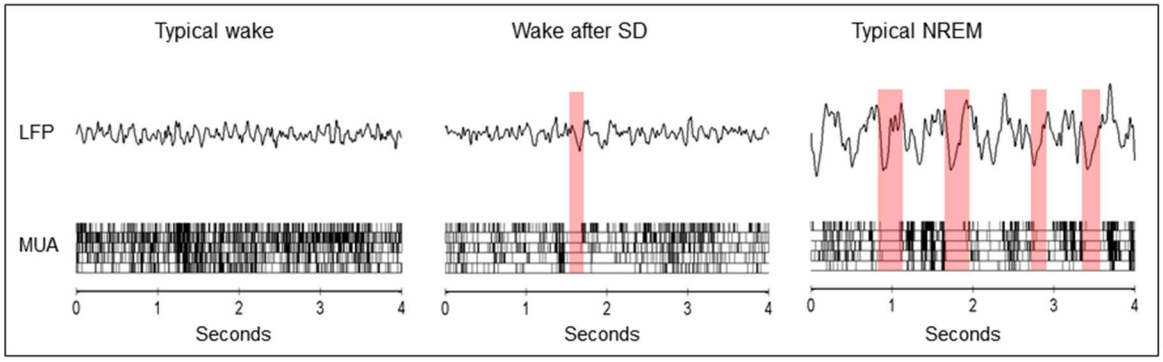

Figure 2

Representative example of the MUA pattern and the related LFP. Left panel: during wakefulness cortical neurons are continuously active giving rise to a fast, low amplitude LFP. Middle panel: with increasing sleep pressure (*i.e.* under sleep deprivation), intermitted brief off-states occur, associated with a theta wave in the LFP. Right panel: the slow oscillation during NREMS associated with sleep slow waves in the LFP (off-states are indicated by the red bars, data provided by Vyazovskiy).

So far, characteristics of sleep homeostasis in the EEG, and their underlying neuronal activity patterns, during wake and sleep have been highlighted. In sum, during the last years we have gained important insights into the electrophysiological mechanisms underlying sleep regulation. By combining intracortical and surface EEG recordings, different markers in the surface EEG were found to be related to underlying neuronal activity patterns. Thus, these markers allow to track the regulation of sleep homeostasis on the cellular level, non-invasively in the EEG in humans. However, despite this profound knowledge about the electrophysiological mechanism of sleep homeostasis, its function remains elusive. Hence, different hypotheses have been formulated during the past years, of which two will be presented in the next section.

3.2 Two Hypotheses for the function of NREM sleep

All of us know the recuperative effect of a good night of sleep. It is unquestionable that sleep is required for normal physiological functioning. Without sleep, our body suffers serious consequences on many different levels including impaired cognitive functioning (Cirelli and Tononi, 2008; Van Dongen et al., 2003), negative effects on metabolism, the immune system, or hormone balance (Xie et al. 2013; Guyon et al. 2014; Tasali et al. 2008; Copinschi et al. 2014). All these impaired functions are restored during sleep. However, what exactly is being restored by sleep remains abstract. In the following, two different hypothesis for the function of NREMS will be presented: *"SHY"* proposes that sleep is the price for brain plasticity (Tononi and Cirelli, 2014) and *"sleep for the single neuron"* suggests that sleep is necessary for neuronal maintenance (Vyazovskiy and Harris, 2013).

3.2.1 Synaptic homeostasis hypothesis (SHY)

SHY was described for the first time in 2003 (Tononi and Cirelli, 2003) and is one of the most prominent hypothesis suggesting a causal role of sleep for brain plasticity and learning. SHY proposes that sleep is required to re-establish synaptic homeostasis which is challenged by the use-dependent increase of synaptic strength during wakefulness and synaptogenesis during development (Tononi and Cirelli, 2014). Over the past years, various studies on the molecular, electrophysiological and structural level have been performed, providing profound evidence that net synaptic strength is tightly regulated across 24-hours: it increases during wakefulness and decreases during sleep (Bushey et al., 2011; Gilestro et al., 2009; Maret et al., 2011; Vyazovskiy et al., 2008). According to SHY, environmental interactions and learning during wakefulness trigger plastic changes of neuronal circuits, mainly by synaptic potentiation and the formation of new synapses, thereby increasing synaptic strength. Such an increase in synaptic strength results in neuronal burdens such as increased energy consumption and cellular stress, greater demand for cellular supply and associated functions of supporting cells. Moreover, it reduces the selectivity of neuronal responses to different stimuli and saturates learning capacity. Therefore, neurons must finally renormalize total synaptic strength (*i.e*. synaptic downscaling) in order to restore neuronal selectivity and cellular functions (for review Tononi and Cirelli, 2014). SHY proposes an activity-dependent downscaling of synaptic strength which takes place during slow wave sleep, when the brain is disconnected from the environment and not disturbed by external stimuli: Synaptic strength is proportionally reduced, while synapses ending up under a certain threshold will be completely eliminated. Thus, synapses which were strengthened repetitively during the day remain after sleep whereas synapses which were less important are eliminated. As learning primarily happens via the repeated strengthening of synapses during the day, the proportional downscaling ensures that important information acquired during waking will be preserved, while unimportant information will be deleted. Thereby, cellular functioning and neuronal selectivity can be restored and the signal-to-noise ratio will be increased, enabling improved performance the next day. One important corollary of SHY is that synaptic downscaling is reflected in sleep slow waves during NREMS. Thus, the homeostatic regulation of sleep mirrors the homeostatic regulation of synaptic strength. Based on SHY, increasing synaptic strength during wakefulness facilitates the highly synchronized activity pattern of the slow oscillation between cortical neurons which is associated with high amplitude slow waves at the beginning of the night. By contrast, synaptic downscaling during sleep reduces the level of synchronization which is

necessary to produce high amplitude slow waves, leading to reduced SWA towards the end of the night (Vyazovskiy et al., 2009). Indeed, there are various experimental data in animals and humans supporting this notion. For example, a steeper slope of the cortical evoked potentials by electrical stimulation after prolonged wakefulness (which is a marker of increased synaptic strength) correlates with SWA at the beginning of the subsequent sleep episode in rats (Vyazovskiy et al., 2008). Moreover, elevated BDNF level (which is a marker of synaptic potentiation) in rats due to being exposed to an enriched environment is associated with increased SWA during subsequent sleep (Huber et al., 2007). In humans, high-density EEG recording revealed local use-dependent changes of SWA. After performing a visuo-motor learning task, SWA was increased over the area which is mainly involved in the task (Huber et al., 2004). On the other hand, SWA was reduced over contralateral somatosensory cortex following arm immobilization (Huber et al., 2006). Moreover, it has been suggested that the dynamic of synaptic downscaling in the course of sleep can be depicted by the slope of slow waves (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007b). As described above, synaptic strength is assumed to be reflected in the level of synchronized neuronal network activity. During the slow oscillation, the slope of slow waves best reflects how synchronously the transition from on- to off-state across neuronal populations occurs (Vyazovskiy et al., 2009). Thus, under high synaptic strength at the beginning of the night, the slope of slow waves is much steeper compared to the slope of slow waves with the same amplitude towards the end of the sleep episode after synaptic downscaling has occurred (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007b). The second important statement of SHY is that sleep slow waves are not only an epiphenomenon of synaptic homeostasis, but also actively contribute to synaptic downscaling. The hypothesis claims that the firing pattern of the slow oscillation underlying sleep slow waves are preferably suited to reduce synaptic strength either by long-term depression (LTD) of excitatory postsynaptic potentials (EPSP; Czarnecki et al. 2007) or by decoupling through synchrony (Lubenov and Siapas, 2008).

In a nutshell, SHY proposes a framework in which the observed changes in the firing pattern of cortical neurons owing to the sleep-wake history are linked to changes in synaptic strength. However, up to date, there is no method available to measure directly synaptic strength in humans. Nevertheless, during the last decades, a large body of translational research on the synaptic, single cell, network and computational level has built a bridge to relate changes in network connectivity to different EEG markers (for review see Tononi & Cirelli 2014). In accordance with these assumptions, changes in network connectivity can be tracked in the human surface EEG: In the spontaneous EEG, such changes are best reflected by the slope of slow waves which mirrors the level of neuronal firing synchrony (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2008). Yet, another possibility to record changes in synaptic strength in humans is by assessing changes in cortical excitability, which can be measured by transcranial magnetic stimulation (TMS, Esser et al., 2006).

3.2.2 Sleep for the single neuron

Only recently, a complementary hypothesis for the fundamental function of sleep has been proposed by Vyazovskiy and Harris (2013). Compared to SHY, which relates sleep to synaptic homeostasis, this hypothesis places the “off-states” underlying slow wave sleep at the centre. The authors suggest that

the most important role of NREMS is to offer single neurons a time window (*i.e.* off-states) to repair minor cellular wear and tear which accumulates during wakefulness before cellular damage becomes irreversible. This preventative process is thought to preclude major impacts on neuronal functioning. In view of this hypothesis, in early waking, cortical neurons are continuously active, firing at their total capacity. In the course of being fully active, the neuron's capacity reaches its limit. This results in multiple forms of cellular stress including the production of reactive oxygen species (ROS), vesicle damage due to repetitive cycling, or protein misfolding in the endoplasmic reticulum (Kourtis and Tavernarakis, 2011; Kültz, 2005; Walter and Ron, 2011). The accumulation of such cellular burdens triggers a stress response allowing the neurons to protect themselves from irreversible neuronal damage. Similar to skeleton muscle cells, which undergo a temporary decline in performance, a process known as muscle fatigue (Allen et al., 2008), cortical neurons experience "neuronal fatigue" *i.e.* cessation of neuronal firing which manifests as intermitted brief local off-states (Rechtschaffen, 1998). Such off-states offer neurons a time period to perform different processes of cellular maintenance including degradation and replacement of waste products, re-allocation of energy substrates, and biosynthesis of new cellular proteins and lipids, such as vesicles and other membrane components (Burré et al., 2010; Denker and Rizzoli, 2010). However, these off-states are not stable: inputs arising during an off-state immediately evoke neuronal response and, thus, terminate the off-state (Petersen et al., 2003; Vyazovskiy et al., 2012). As a consequence of the extensive interconnections of cortical neurons, off-states during waking last only a few milliseconds due to high background network activity (Léger et al., 2005; Piantoni et al., 2013). Therefore, short-lasting off-states during waking might serve as a protective mechanism of cortical neurons to prevent further accumulation of cellular wear and tear. They are, however, not efficient enough to restore cellular homeostasis. In addition, reliable processing of sensory information might be impaired in neurons which undergo off-states during waking, potentially impacting behaviour and performance (Harris and Thiele, 2011; Pigorini et al., 2015; Sachdev et al., 2004; Vyazovskiy et al., 2012). In conclusion, prolonged off-states can only occur when large neuronal populations engage in off-states simultaneously, a state which is not compatible with adequate performance during waking. Thus, efficient cellular maintenance requires synchronized network activity and withdrawal from environmental inputs. This is the established manifestation of sleep. The prolonged off-states during stable slow waves sleep provide single neurons with a time period, to restore cellular homeostasis during NREMS. Indeed, many of the genes known to be upregulated during sleep are involved in different restorative functions such as protein synthesis, membrane trafficking and cellular maintenance (Cirelli et al., 2004). However, the notion that neuronal off-states arise only as a consequence of neuronal fatigue due to excessive firing has been challenged by very recently published results (Rodriguez et al., 2016). These authors observed that a wake-like local increase in neuronal activity obtained by optogenetic stimulation during NREMS in mice did not induce longer or more frequent off-states during subsequent sleep after stimulation. Thus, only neuronal activity occurring in the metabolic, neuromodulatory and neurochemical milieu of active wake seems to be associated with an increasing need for sleep.

In summary both hypothesis, "SHY" and "sleep for the single neuron" represent a corticocentric view for the function of NREMS, putting sleep slow waves on central stage. For both hypotheses, several lines

of evidence exist and the assumption of the two hypothesis are not exclusive. However, most of the studies performed in humans so far are descriptive and do, therefore, only provide correlative evidence. Thus, selective manipulation of sleep slow waves is needed to further establish a causal role of sleep slow waves on the restorative function of both synaptic and neuronal homeostasis.

3.3 Manipulation of sleep slow waves

Considering the pivotal role of slow waves for the recuperative function of sleep, it is not surprising that several strategies have been explored to increase slow waves during sleep. For example, slow waves can be pharmacologically enhanced using either gaboxadol (a GABA agonist), or tiagabine (a GABA reuptake inhibitor) which are associated with reduced daytime sleepiness and improved memory performance, respectively (Walsh et al., 2008, 2006). Even though these results are promising, pharmacological interventions bear the risk for developing tolerance and dependence. Therefore, other methods have been investigated to increase slow waves through non-pharmacological means. One possibility which has been proven to be effective in enhancing slow waves is stimulating the brain with electrical currents or magnetic fields. Marshall and colleagues (2006) successfully increased the low frequency portion of SWA by intermittent transcranial direct-current stimulation (tDCS). Strikingly, this increase was associated with improved performance in a declarative memory task. However, the direct effect on neuronal activity through tDCS cannot be assessed in the EEG due to the strong electrical artefacts during stimulation. Yet another option to non-invasively enhance slow waves in human is by applying transcranial magnetic stimulation (TMS) on the scalp during NREMS (Massimini et al., 2007). In contrast to tDCS, the direct EEG response to the TMS pulse can be analysed: TMS pulses emitted at the right frequency are able to trigger a full-fledged slow wave that starts under the coil and spreads across the cortex (Massimini et al., 2007). However, due to practical reasons (*i.e.* high cost, challenging implementation), TMS is not applicable for long-term application. Hence, other research has focused on the possibility to manipulate slow waves in a more natural manner using physiological sensory input. Among the different somatosensory modalities, auditory stimulation has been shown to be particularly efficient in enhancing slow waves in humans (Bellesi et al., 2014; Tononi et al., 2010). Acoustic stimulation during NREMS allows to manipulate slow waves in both direction, it either enhances (Arzi et al., 2012; Ngo et al., 2013; Riedner et al., 2011) or disturbs slow waves (Aeschbach et al., 2008; Landsness et al., 2009; Ngo et al., 2013). These two kinds of manipulation seem to be associated with improved (Arzi et al., 2012; Ngo et al., 2013) and impaired performance, respectively (Aeschbach et al., 2008; Landsness et al., 2009) the next day. However, these observed effects of auditory stimulation on sleep slow waves were accompanied by other changes in the sleep EEG, which limits claims about causal relationship between sleep slow waves and the detected performance changes. For example in the study of Ngo and co-authors, auditory stimulation also increased fast spindle power (Ngo et al., 2013). In the studies where slow waves were perturbed with auditory stimulation a pronounced portion of time spent in N3 sleep was shifted to N2 sleep, thereby interfering with the overall structure of sleep architecture. As a result sleep propensity during the day was increased which has negative consequences on daytime performance (Aeschbach et al., 2008; Landsness et al., 2009). Consequently, a more selective manipulation of sleep slow waves is needed to establish causality between sleep slow waves and the observed electrophysiological and behavioural consequences. Only

recently it has been discovered that the phase of the ongoing slow oscillation plays an important role for the fates of incoming auditory stimuli (Ngo et al., 2013). Stimulation time-locked to the up-phase of the slow waves (*i.e.* the on-state, this is when neurons are depolarized) seems to induce a fast and efficient synchronization of neuronal activity with increased neuronal spiking. Given the activity-dependent nature of the I_{DK} (as described in section 3.1.1) such increased activation during the on-state might result in more pronounced neuronal hyperpolarization (Hill and Tononi, 2005). On the EEG level, this would result in enhanced SWA, *i.e.* slow waves with increased amplitudes and steeper slopes (Bellesi et al., 2014). On the other hand, stimulation time-locked to the down-phase of sleep slow waves (*i.e.* the off-state, this is when neurons are hyperpolarized) might have a disruptive effect on the following slow wave (Ngo et al., 2013). Probably due to altered membrane properties (*e.g.* a larger glutamatergic driving force) during hyperpolarization, incoming stimuli are likely to provoke neuronal firing (Sachdev et al., 2004), resulting in a premature termination of the off-state. Considering the strong interconnection of cortical neurons, such induced neuronal activity might interfere with the ongoing slow oscillation in the network, thereby reducing the level of synchronization which is necessary to produce high amplitude slow waves. Beside the phase-timing of auditory stimulation, also the intensity of the stimuli plays an important role. Due to the different arousal levels, depending on sleep depth, the volume should be continuously adjusted to prevent arousals or fragmentation of sleep. Thus, presenting acoustic stimulation at inappropriate volume or inappropriate time could induce undesired non-specific effects on sleep. In this respect, the ability to adjust timing and intensity of the stimulation, moment by moment according to the ongoing brain activity becomes absolutely crucial. Such dynamic control can be realized by developing closed-loop systems allowing to continuously monitor the EEG online, and detecting slow waves in real-time (Bellesi et al., 2014).

Heretofore, the principle underlying mechanisms and possible related functions of sleep homeostasis and how these might be selectively investigated have been summarized. If sleep slow waves fulfil important function of synaptic regulation then they should do so even more prominently during development. Human brain development (*i.e.* from infancy up to and including adolescence) represents a time window, when the brain is extremely plastic, undergoing massive changes in cortico-cortical connectivity. Hence, EEG recordings during this time window offer an ideal opportunity to investigate different electrophysiological markers during waking and sleep related to changes in network connectivity and sleep homeostasis. Thus, in the following section, the maturation of the human brain from early postnatal life until adulthood will be introduced.

3.4 Development of the human brain

During brain maturation, the human brain undergoes massive morphological changes, which are paralleled by the development of cognitive functions (Gogtay et al., 2004; Johnson, 2001). These changes are accompanied by different electrophysiological markers in the wake and sleep EEG.

3.4.1 Morphological changes during brain development

Post-mortem studies of the human brain revealed that massive formation of new synapses occurs during the first months of postnatal life (Huttenlocher, 1990). Thereby, synapse density peaks first in the visual

cortex during infancy and early childhood whereas peak synaptic density in more frontal brain areas is reached during late childhood and adolescence (Huttenlocher and Dabholkar, 1997). This substantial increase of synaptic formation during childhood is followed by a distinct reduction of synapse density during puberty, a process termed "synaptic pruning" (Huttenlocher, 1978). These findings were confirmed *in vivo* by measuring cortical grey matter volume (*i.e.* an indirect marker of synaptic density) using magnetic resonance imaging (MRI). Grey matter volume increases in the first year of life, reaching a maximum shortly before puberty, followed by a decline throughout adolescence. Moreover, this maturation process seems to occur first over posterior regions and shifts to anterior regions in the course of development (Giedd, 2004; Shaw et al., 2008). Compared to this u-shaped developmental trajectory of grey matter volume, white matter maturation follows a linear increase during development, not levelling off until the 30s (Paus et al., 2001).

3.4.2 Electrophysiological markers of brain plasticity and sleep homeostasis during development

According to SHY, SWA during NREMS reflects synaptic strength (Tononi and Cirelli, 2014). Considering this massive changes in synaptic density during brain development, comparable maturational changes in SWA are expected. Indeed, as synaptic density SWA follows a similar inverted U-shape across development reaching peak SWA values shortly before puberty (Campbell and Feinberg, 2009; Feinberg, 1982; Gaudreau et al., 2001). Moreover, using hd-EEG in a cross-sectional study of children and adolescents between 2 and 20 years of age, Kurth et al., (2010) showed predominant SWA shifts from posterior to anterior regions with age, presumably reflecting the regional trajectory of brain maturation (Kurth et al., 2010b). Considering this observation, SWA might indeed mirror cortical maturation during development. Nevertheless, these developmental changes of synaptic strength occur on a large time scale. What about sleep homeostasis, *i.e.* the homeostatic changes in synaptic strength on a daily bases during development? How does the massive formation of new synapses during the first year of life effect daily sleep regulation? Interestingly, during the first year of life, SWA does not seem to reflect sleep homeostasis. Compared to the well-known decline of SWA across sleep cycles found in adults, SWA exhibits an alternating pattern in infants, with a high amount of SWA in every other sleep cycle (Jenni et al., 2004). This finding triggers the question whether SWA may not be a suitable marker to detect sleep homeostasis, or whether the homeostatic regulation of sleep is not yet established during early postnatal live. As highlighted in section 3.1.2, sleep homeostasis in not only mirrored by SWA but can also be mapped on the level of synchronized neuronal activity among cortical neurons (Vyazovskiy et al., 2009). The level of network synchronization seems to be best reflected in the slope of slow waves (Esser et al., 2007; Vyazovskiy et al., 2009). Hence, the slope of slow waves might serve as an alternative marker for sleep homeostasis during infancy.

On the other hand, during late childhood, the time when synaptic density reaches its maximum, the build-up of sleep pressure during waking might be very strong. This assumption is in line with the finding that the build-up of sleep pressure during the day seems to be faster in pre- to early pubertal children compared to adults (Carskadon et al., 2004; Jenni et al., 2005). Moreover, a forward shift of the circadian rhythm at this age has been described, which might further augment high sleep pressure in the evening (Carskadon et al., 2004; Hagenauer et al., 2009; Jenni et al., 2005). Thus, compared to adults, such

increased drive of sleep homeostasis during the day, in pre- to early pubertal children, might enhance theta activity (*i.e.* the electrophysiological signs of intermitted brief off-states) in the evening wake EEG (*i.e.* after normal waking hours).

So far, the EEG measures introduced in this work were based on either power values or wave characteristics, *i.e.* the slope and the amplitude. Yet, there are also other possibilities to quantify the sleep and wake EEG. For example, information based measures, such as approximate entropy (ApEn), are focusing on the reduction of consciousness associated with NREMS. Such a reduced consciousness level might be due to reduced long-distance effective connectivity during NREMS compared to REMS and wakefulness (Massimini et al., 2007, 2005). More specifically, ApEn is a measure of regularity in the time domain. It quantifies the predictability of a signal by calculating the number of matching sequences of a given length of units with the number of matching sequence one unit longer (Pincus, 1991). A reduction of ApEn is thought to reflect a breakdown of long-distance connectivity (Pincus, 1994) and has therefore been suggested as a measure to asses different vigilance states (Burioka et al., 2005). The morphological development of brain structure, *i.e.* the pruning of excrescent synapses during puberty and the parallel development of white-mater structure are accompanied by a reorganization of neuronal circuits to develop more specific and meaningful cortical networks (Lichtman and Colman, 2000). Thus, refinement of cortical synapses, together with a linear increase of white-mater maturation might increase efficient information transfer to improve the functional integration of distant brain regions (Fair et al., 2007; Tarokh and Carskadon, 2010). Hence the question arises whether these maturational changes are also depicted in ApEn values during wake and sleep across development.

In summary, either in the long (*i.e.* over years) or the short (diurnal time period) run, the development from infancy to adulthood provides an exclusive time-window to investigate electrophysiological markers related to alterations in network connectivity, owing to vast adaptations of the grey and white matter in the human brain. However, what happens if slow wave sleep during this crucial time period is disturbed, for example in children diagnosed with a childhood epilepsy characterized by an activation of epileptic activity during slow wave sleep?

3.4.3 Childhood epilepsies characterized by an activation of spike waves during slow wave sleep

In some childhood epilepsies, the epileptiform abnormalities in the EEG are particularly pronounced during slow wave sleep and these abnormal neuronal activity patterns are thought to contribute to a disturbance in cerebral functioning, as the onset of the epilepsy is often associated with developmental arrest or regression (ILAE, 1989). Another important attribute of these epilepsies is that they occur only during a specific developmental stage and are resolved as development progresses. This might indicate that the emergence of the epilepsy could be related to a specific time window of brain maturation. For example, West syndrome usually arises within the first year of live. It is characterized by infantile spasms, frequent mental retardation and an abnormal EEG pattern known as hypsarrhythmia (*i.e.* random, high voltage spikes and slow waves with variable amplitudes, for an example see Figure 3, lower panel), which is most prominent during slow wave sleep (Hancock et al., 2009). Another example is the epilepsy with continuous spike waves during sleep (CSWS), which occurs during childhood, the

age when synaptic density is highest. Also, children diagnosed with CSWS have variable forms of neurophysiological impairments, which are associated with continuous diffuse spike-waves during slow wave sleep in the EEG (for example see Figure 3, middle panel, ILAE 1989; Caraballo et al. 2013).

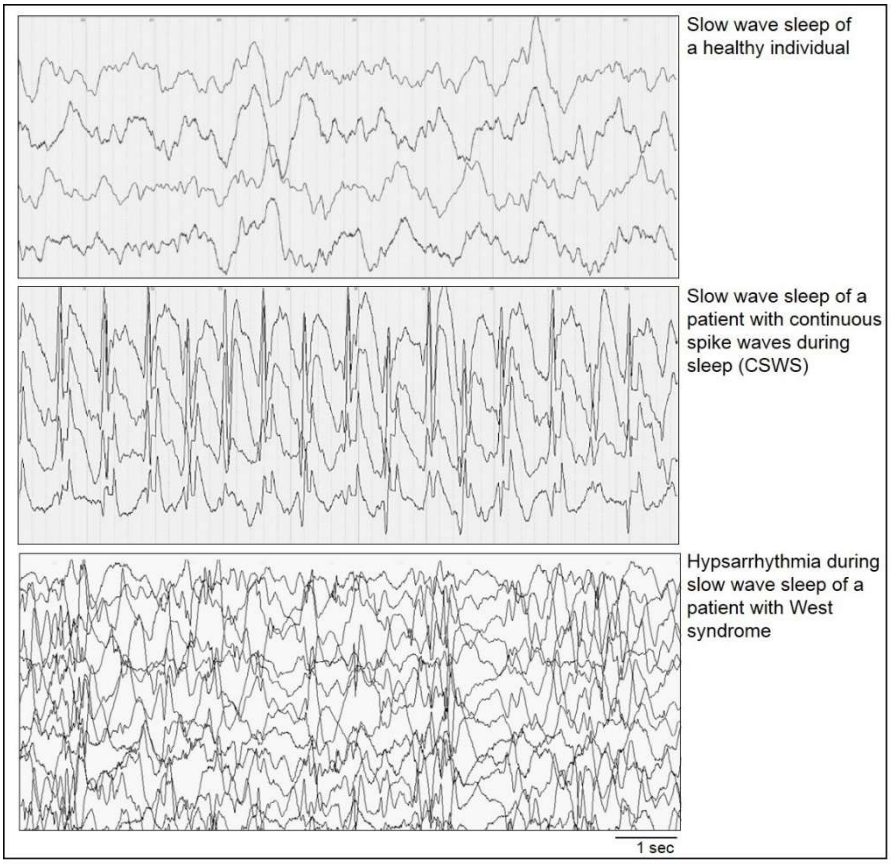

Figure 3

Representative examples of EEG activity during slow wave sleep of a healthy individual, and of two patients with a childhood epilepsy characterized by an activation of spike waves during slow wave sleep. Upper panel: The EEG of a healthy individual displays synchronized high amplitude slow waves. Middle panel: The EEG of a patient diagnosed with continuous spike waves during sleep (CSWS). The EEG displays continuous diffuse spike-waves. Lower panel: The EEG of a patient diagnosed with West syndrome. The EEG displays the chaotic pattern of hypsarrhythmia, characterized by random, high voltage spikes and slow waves with variable amplitudes.

As shown in Figure 3, slow wave sleep is tremendously altered in children diagnosed with either West syndrome or CSWS. Considering the important role of physiological slow waves to keep up synaptic and neuronal homeostasis, the question arises whether these restorative functions can be fulfilled even under altered slow wave conditions. Or might these epileptiform abnormalities perturb physiological slow waves, which could account for the observed deterioration of mental developmental at epilepsy onset? In a recent retrospective analysis, Bölsterli et al., reported an impaired overnight reduction of the slope of slow waves in children diagnose with CSWS (Bölsterli et al., 2011), providing first evidence that the regulation of sleep homeostasis might be disturbed in these children. The interpretation of this finding was that epileptiform discharges, *i.e.* spike waves during slow wave sleep, might disturb the

physiological function of slow waves. However, the authors did not relate spike wave activity to the observed impaired overnight reduction of the slope of slow waves. Thus, additional studies are needed to further elaborate whether the pathological epileptiform brain activity accounts for the impaired renormalization of the slope of slow waves after sleep. If so, one would expect, for example, that the impaired renormalization of the slope of slow waves is related to the localization and density of spike waves. Or that successful treatment of the epileptiform abnormalities in the EEG would be accompanied by a normalization of the overnight dynamics of the slope of slow waves.

3.5 Aims of this thesis

The overall aim of the current thesis was to investigate the causal function of sleep slow waves on synaptic homeostasis in humans. In a first step, we elaborated different electrophysiological markers of network connectivity to map sleep homeostasis in the human wake and sleep EEG. To do so, we first performed descriptive analyses by retrospectively analysing different features in the sleep and wake EEG during development, the time when maximal changes in cortico-cortical connectivity occur. To further explore the role of sleep slow waves in the regulation of synaptic homeostasis, we focused on the sleep-dependent decrease of network synchronization *i.e.* the overnight dynamic of the slope of slow waves. In a second step, we investigated the overnight reduction of the slope of slow waves in a clinical population with disturbed slow wave sleep, *i.e.* children diagnosed with West syndrome or CSWS. To further shed light on the causal function of slow waves on synaptic homeostasis, in the third and last step of the current thesis, we developed a closed-loop auditory stimulation tool, to selectively manipulate slow waves during NREMS. This tool was then tested, combined with TMS and behavioural measurements in a prospective study in young healthy adults, to evaluate the causal role of sleep slow waves on cortical excitability and performance.

3.5.1 Different electrophysiological markers of sleep homeostasis and network connectivity in the sleep and wake EEG during development

In three retrospective analyses, we established the following indirect electrophysiological markers of network connectivity and sleep homeostasis in human sleep and wake EEG: (1) the slope of slow waves to map the physiological reduction of network synchronization during sleep already in early postnatal life, (2) widespread theta events in the wake EEG to map the occurrence of short intermitted local off-states in the wake EEG in children (3) and ApEn in the wake and sleep EEG in children and adults to map maturational changes in long distance network connectivity during development.

1) The overnight reduction of the slope of slow waves might mirror sleep homeostasis during infancy

During early postnatal brain development, a more sensitive marker of network synchronization than SWA might be necessary to map minor overnight changes in network connectivity. Thus, our aim was to investigate whether the slope of sleep slow waves might provide an alternative to study the homeostatic regulation of sleep during early human development.

Research article: *Overnight Changes in the Slope of Sleep Slow Waves during Infancy*

Sara Fattinger, Oskar G. Jenni, Bernhard Schmitt, Peter Achermann, Reto Huber, (Fattinger et al. 2014 in *Sleep*)

2) Widespread theta events in the wake EEG of children might reflect local intermitted off-states in a subset of cortical neurons

During late childhood, the age when synaptic density is highest, the build-up of sleep pressure during wakefulness might be very strong, resulting in high levels of sleep pressure in the evening. Such increased sleep pressure might induce intermitted brief off-states (*i.e.* local sleep) in a subset of cortical neurons which can be detected as theta waves in the surface EEG (Vyazovskiy et al., 2011). Indeed, in a preliminary analysis we found a pronounced increase of theta activity from morning to evening in pre- to early pubertal children. Based on this finding, we aimed at investigating whether theta waves in the waking EEG fulfil signs of local sleep in children.

Research article: *Oscillatory theta events in the waking electroencephalogram of children resemble local aspects of sleep during wakefulness*
Sara Fattinger, Salome Kurth, Maya Ringli, Oskar G. Jenni, Reto Huber (submitted)

3) Approximate entropy (ApEn) in the sleep and wake EEG might map maturational changes in long distance connectivity during development

Information based measures, such as ApEn, are thought to reflect effective long-distance connectivity. The refinement of cortical synapses during adolescence (Lichtman and Colman, 2000) accompanied with a linear increase of white-mater maturation, might improve the functional integration of information processing of distant brain regions (Fair et al., 2007; Tarokh and Carskadon, 2010). Hence, we aimed at investigating whether these maturational changes are also depicted in ApEn values during wake and sleep across development.

Research article: *Electroencephalogram approximate entropy influenced by both age and sleep*
Gerick Lee, **Sara Fattinger**, Anne-Laure Mouthon, Quentin Noirhomme, Reto Huber (Lee et al. 2013 in *Frontiers in Neuroinformatics*)

3.5.2 Relationship of the overnight reduction of the slope of slow waves with epileptic activity during sleep in CSWS and West syndrome patients

In the second step, we focused on the role of sleep slow waves in the regulation of synaptic homeostasis. The overnight reduction of the slope of slow waves is thought to reflect the physiological sleep related decrease of synaptic strength (Tononi and Cirelli, 2014). Slow wave sleep is severely altered in children diagnosed with CSWS or West syndrome. Assuming that physiological slow waves are causally involved in synaptic downscaling, the overnight reduction of the slope of slow waves is expected to be impaired in these patients. Such impaired reduction of the slope of slow waves has previously been reported in CSWS patients (Bölsterli et al., 2011). In two follow-up studies we further elaborated the role of epileptiform discharges on such impaired overnight reduction of the slope of slow waves. In two different

retrospective analysis, we aimed at investigating the overnight dynamic of the slope of slow waves and its relationship first to spike wave activity and second to successful treatment.

4) Impaired overnight decrease of the slope of slow waves is related to location and density of spike waves

Children diagnosed with CSWS show an impaired overnight reduction of the slope of slow waves compared to healthy age and gender matched controls (Bölsterli et al., 2011). In a further study we wanted to investigate whether such impaired overnight reduction of the slope of slow waves is related to the epileptiform abnormalities in the EEG (*i.e.* the spike wave index).

Research article: *Spike wave location and density disturb sleep slow waves in patients with CSWS (continuous spike waves during sleep)*

Bigna Bölsterli Heinzle**, Sara Fattinger**, Salome Kurth, Monique LeBourgeois, Maya Ringli, Thomas Bast, Hanne Critelli, Bernhard Schmitt, Reto Huber, (Bolsterli Heinzle et al. 2014 in *Epilepsia*)

5) Successful treatment of hypsarrhythmia in West syndrome patients might be related to a normalization of the slope of slow waves

As in CSWS, also in West syndrome patients, physiological slow wave sleep is severely altered in the EEG due to hypsarrhythmia. Hypsarrhythmia might be effectively treated with hormone therapy (Adrenocorticotropic hormone (ACTH) or corticosteroids) or vigabatrin (VGB). In the current study, we investigated whether the physiological overnight reduction of the slope of slow waves is also impaired in West syndrome patients, and if so, how the slope of slow waves changes under and after successful treatment of hypsarrhythmia.

Research article: *Impaired slow wave sleep downscaling in West syndrome – a severe epileptic encephalopathy*

Sara Fattinger, Bernhard Schmitt, Bigna Bölsterli Heinzle, Hanne Critelli, Oskar G. Jenni, Reto Huber (Fattinger et al. 2015 in *European Journal of Paediatric Neurology*)

3.5.3 Selective slow wave manipulation to assess a causal function of sleep slow waves on synaptic homeostasis in humans

In the third and last step of the current thesis project, we developed a closed-loop auditory stimulation tool to detect sleep slow waves in real time. With this tool, we sought to selectively manipulate sleep slow waves locally, in one specific brain area, without interfering with the global structure of sleep. The functionality of our approach was based on the following three important assumptions of EEG sleep slow waves in humans which were previously introduced: (1) slow waves are locally regulated, (2) the down-phase of slow waves in the EEG corresponds to the silent off-states of the underlying slow oscillation and (3) the phase of the ongoing oscillation of slow waves plays an important role for the provoked response of the incoming stimuli: stimulation time-locked to the up-phase of slow waves seems to trigger slow waves whereas stimulation time-locked to the down-phase seems to disturb the ongoing slow oscillation. To assess local changes of slow wave manipulation due to auditory stimulation,

we combined the closed-loop auditory stimulation tool with hd-EEG recording. Thus, slow waves were detected in real time in one specific brain area (*i.e.* by selecting one specific electrode of the hd-EEG net), and tones were played precisely time-locked to the down-phase of the detected slow waves to disturb the ongoing slow oscillation in the selected brain area. As slow waves are locally regulated, the tones are only systematically time-locked to the down-phase of the slow waves in the selected brain area while in the remaining brain areas, tones will be randomly presented at any phase of the ongoing slow waves. With this approach, we aimed to induce a local reduction of SWA over the selected brain area to assess the causal function of sleep slow waves in humans. The tool was tested in a prospective study in young healthy adults.

6) Local slow wave perturbation is related to impaired sleep-dependent recovery processes in humans

To establish a causal function of sleep slow waves on synaptic homeostasis in humans, one needs to selectively manipulate sleep slow waves and assess the resulting consequences on synaptic strength. Thus, in the current study, we combined local slow wave perturbation during sleep in the primary motor cortex, using our closed-loop auditory stimulation tool, and TMS measures to assess the consequences on behavioural and neurophysiological markers of neuroplasticity arising from a dedicated motor practice. To do so, an established TMS paradigm which allows to record use-dependent changes in cortical excitability due to a finger taping sequence task was applied in the morning, evening and again the next morning after one night of sleep, which was recorded using hd-EEG. In a counterbalanced cross-over study design, subjects participated twice. During one night, subjects had a normal night of sleep while during the other night, slow waves were locally disturbed over the left primary motor cortex. To establish a causal function of sleep slow waves on synaptic homeostasis, the resulting consequences of local slow wave perturbation on cortical excitability of the left primary motor cortex and performance in the finger tapping sequence task were quantified.

Research article: *Deep sleep maintains learning efficiency of the human brain*
Sara Fattinger*, Toon T. de Beukelaar*, Kathy L. Ruddy*, Carina Volk, Natalie C. Heyse, Joshua A. Herbst, Richard Hahnloser, Nicole Wenderoth**, Reto Huber** (resubmitted to *Nature Communications*)
*/** equal contribution

In the following chapter, the research articles as listed above, are presented.

4. Research Articles

4.1 Overnight Changes in the Slope of Sleep Slow Waves during Infancy

Sara Fattinger, MS[1,2]; Oskar G. Jenni, MD[1,2,5]; Bernhard Schmitt, MD[2,3]; Peter Achermann, PhD[4,5,6]; Reto Huber, PhD[1,2,5,6]

[1] Child Development Center, University Children's Hospital Zurich, Switzerland;
[2] Children Research Center, University Children's Hospital Zurich, Switzerland;
[3] Division of Clinical Neurophysiology, University Children's Hospital Zurich, Switzerland;
[4] Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland;
[5] Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland;
[6] Neuroscience Center Zurich (ZNZ), University of Zurich, Zurich, Switzerland

Financial support was provided by the Swiss National Science Foundation grants 3100-05300.97, 3100A0-100567, 320030-130766 and PP00A-114923, the Human Frontiers Science Program Grant RG 0131/2000-BR102 and a research grant from the University Research Priority Program of the University of Zurich.

Own contribution: analysed the data, wrote and edited the manuscript

Published in
Sleep 2014, 37(2): 245-253

Abstract

Study objectives: Slow wave activity (SWA, 0.5-4.5 Hz) is a well-established marker for sleep pressure in adults. Recent studies have shown that increasing sleep pressure is reflected by an increased synchronized firing pattern of cortical neurons, which can be measured by the slope of sleep slow waves. Thus we aimed at investigating whether the slope of sleep slow waves might provide an alternative marker to study the homeostatic regulation of sleep during early human development.

Design: All-night sleep electroencephalography (EEG) was recorded longitudinally at 2, 4, 6, and 9 months after birth

Setting: home recording

Patients or participants: 11 healthy full-term infants (5m, 6f)

Interventions: None

Measurements and results: The slope of sleep slow waves increased with age. At all ages the slope decreased from the first to the last hour of NREM sleep, even when controlling for amplitude differences ($P < 0.002$). The decrease of the slope was also present in the cycle-by-cycle time course across the night ($P < 0.001$) at the age of 6 months when the alternating pattern of low-delta activity (0.75 - 1.75 Hz) is most prominent. Moreover, we found distinct topographical differences exhibiting the steepest slope over the occipital cortex.

Conclusions: Our results suggest an age dependent increase in synchronization of cortical activity during infancy, which might be due to increasing synaptogenesis. Previous studies have shown that during early postnatal development synaptogenesis is most pronounced over the occipital cortex, which could explain why the steepest slope was found in the occipital derivation. Finally, our results provide evidence that the homeostatic regulation of sleep develops early in human infants.

Key words: sleep, slope of slow waves, homeostasis, infants, development

Introduction

Sleep shows substantial age-related changes during early human development (Bes et al., 1991; Louis et al., 1997; Navelet et al., 1982). In the course of the first year after birth, the proportion of quiet sleep (QS, NREM sleep) increases (Louis et al., 1997; Navelet et al., 1982) and slow wave sleep (SWS, N3) becomes the predominant sleep stage in the first part of the night (Anders and Keener, 1985; Bes et al., 1991; Coons and Guilleminault, 1982; Fagioli and Salzarulo, 1982) Salzarulo and Fagioli proposed that the higher proportion of SWS at the beginning of a sleep period with a subsequent decrease in the course of a night may reflect the nocturnal dissipation of sleep propensity in young infants. They proposed that sleep regulatory mechanisms develop early in human life (Salzarulo and Fagioli, 1992). In adults, these regulatory mechanisms of sleep have been intensively studied using slow wave activity (SWA, EEG power in the delta frequency range, between 0.5-4.5 Hz) as a quantitative measure of slow waves. According to the two-process model of sleep regulation (Borbély, 1982), the level of SWA reflects NREM sleep pressure and is related to the homeostatic regulation of sleep. SWA increases as a function of prior wakefulness and progressively declines during sleep (Achermann and Borbély, 2003). Interestingly, in contrast to the decline of SWS across NREM/ REM sleep cycles found in adults, the ultradian sleep cycle during early development is characterized by an alternating pattern of SWS occurring in every other sleep cycle. This salient feature of SWS has been shown in humans (Bes et al., 1991) and animals (Alfoldi et al., 1990) and was supported by the study of Jenni and colleagues showing that low-delta activity (0.75-1.75 Hz) follows the same pattern in human infants (Jenni et al., 2004) Specifically, NREM sleep episodes with high levels of low-delta activity alternate with NREM sleep episodes showing only minimal levels of low-delta activity across the night (Jenni et al., 2004). The authors found that the alternating pattern was most prominent at age of 6 months, where no decline in low-delta activity across the night was observed. They concluded from their study that low-delta activity may not be a suitable marker for the homeostatic regulation of sleep pressure during early human development.

Recently, we have gained important insights into the electrophysiological mechanisms underlying sleep regulation. On the neuronal level, slow waves are generated by a slow oscillation of neuronal activity between a depolarized on-state when neurons show sustained firing, and a hyperpolarized off-state when neurons are silent (Steriade et al., 1993b). During deep sleep, slow oscillations involve the majority of cortical neurons and become detectable as high amplitude spatially synchronized slow waves in the surface EEG. Using high density EEG recordings it has been demonstrated that slow waves travel across the cortex, originating in a particular location and propagating across the scalp involving more and more populations of cortical neurons (Massimini et al., 2004). These slow oscillations were further investigated using multiunit recordings in the rat, which have shown that changes in sleep pressure directly affect the alternating firing pattern of cortical neurons (Vyazovskiy et al., 2009). With increasing duration of wakefulness, the slow oscillation among large populations of cortical neurons becomes more and more synchronized, presumably due to an increase in cortical connectivity (Tononi and Cirelli, 2006; Vyazovskiy et al., 2009). Such highly synchronized neuronal activity is reflected in high amplitude, steep scalp slow waves contributing to a high amount of SWA. During subsequent sleep, the synchronization of the neuronal firing pattern decreases, possibly due to a decrease in network connectivity, resulting in

lower amplitude waves on the surface electroencephalogram (EEG), which in turn leads to a decrease in the level of SWA (Vyazovskiy et al., 2009). These observations suggest that the level of synchronization among populations of cortical neurons represent a cellular counterpart of the homeostatic regulation of sleep. It has been proposed that the slope of slow waves, measured in the EEG, may represent a marker of this neuronal synchronization (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007b). Thus, the faster neuronal populations of a network are able to synchronize their activity, the steeper the slopes of the slow waves are. Consequently, the slope of slow waves at the beginning of the night when sleep pressure is high is much steeper compared to the slope of slow waves with the same amplitude towards the end of the sleep period when sleep pressure has dissipated (Riedner et al., 2007) Moreover, Kurth et al. showed that the slope of slow waves decreases during adolescence, presumably reflecting the pruning of synapses during puberty (Kurth et al., 2010a).

The early postnatal period of cortical development is characterized by a vast increase of cortical connectivity due to the formation of new synapses (Huttenlocher, 1990) The study by Jenni et al. has shown that low-delta activity reveals a similar age-dependent increase (Jenni et al., 2004). Moreover, anatomical studies have shown that the increase in synapse density occurs first, during infancy and early childhood in the visual cortex, and spreads to more frontal regions during adolescence (Huttenlocher and Dabholkar, 1997).

The aim of this study was to examine whether the considerable regional increase in cortical connectivity during infancy is also reflected by a change of the slope of sleep slow waves. Moreover, we aimed to investigate whether overnight changes in the slope of slow waves are already present during infancy, which would provide an indication for changes in the synchronization of cortical activity in the course of sleep. Therefore, we examined the characteristics of sleep slow waves (amplitude, slope, and incidence) in the EEG of the same infants published by Jenni and coworkers (Jenni et al., 2004) Single slow waves were detected by applying an automatic detection algorithm (for further detail see (Riedner et al., 2007) and methods) and the amplitude (from zero level to the local minimum of the signal; μV), slope (amplitude of the negative half wave divided by the time between local minimum and the subsequent zero crossing; μV/s), and incidence (total number of detected slow waves per hour of NREM sleep) of slow waves were computed (see Fig. 1A). It has been shown that slope and amplitude of slow waves are closely related (Bölsterli et al., 2011; Kurth et al., 2010a). Thus, for an assessment of the slope, independent of amplitude changes, the slope of slow waves with a fixed amplitude (55 μV) were calculated (Bölsterli et al., 2011). Finally, we were interested whether the increase in cortical connectivity during the first year of life would facilitate the propagation of slow waves across the cortex leading to an increase in the number of global slow waves. To address this question, the propagation properties of the detected slow waves were analyzed.

Methods

Subjects and Study Design

We analyzed previously published data from nocturnal polysomnographic sleep recordings carried out longitudinally in 11 healthy (5 boys and 6 girls) full-term infants (Jenni et al., 2004). Subjects were recruited from the Department of Gynecology and Obstetrics at the University Hospital Zurich (Switzerland) and through private sources. All-night sleep polysomnography was recorded longitudinally at 2 weeks (17.9±1.3 days) and at 2 (69.1±22 days), 4 (133.8±2.2 days), 6 (187.9±2.5 days), and 9 (282.0±2.9 days) months after birth at home in their habitual sleep environment following their usual bedtime routines. All infants had regular sleep-wake rhythm and were free of any infections or respiratory diseases at the time of the recording sessions (Jenni et al., 2004).

Written informed consent was obtained from all parents after detailed explanation of the study design and aim. The study protocol was approved by the ethics committee of the University Children's Hospital Zurich (Switzerland) and was performed according to the declaration of Helsinki.

Polysomnography

In each measuring night, EEG (bipolar derivations: F3-C3, F4-C4, C3-P3, C4-P4, P3-O1, P4-O2; referential derivation: C3-A2), electrooculogram (EOG), submental electromyogram (EMG), ECG, and respiratory movements were recorded at 512 Hz (analogue filter: high pass 0.16 Hz, low pass 70 Hz) with a portable polygraphic amplifier system (PS1; Institute of Pharmacology and Toxicology, University of Zurich, Switzerland). The EEG signal was stored after digital low-pass filtering at 30 Hz with a sampling rate of 128 Hz (for details see Jenni et al. 2004). Before each recording session, the parents were contacted by phone to obtain information on the health status and sleep-wake rhythms during the preceding days. The electrodes were attached by the experimenter (Jenni; developmental pediatrician) in the early evening. The prepossessing of the EEG data and scoring of sleep stages is described in detail in the previous work of Jenni et al. (2004) In short: sleep stages were visually scored (referential derivation C3-A2) for consecutive 20-s epochs as quiet sleep (QS) or active sleep (AS, according to Anders and coworkers; Anders et al. 1971) at 2 months of age; subsequent recordings at higher age were scored as NREM (stages 1-4) or REM sleep (Guilleminault and Soquet, 1978). The later scoring criteria resembles those used in adults (Rechtschaffen and Kales, 1968). For reasons of simplification, only the terms NREM sleep (instead of QS) and REM sleep (instead of AS) are used throughout the manuscript.

Data Analysis

For the current analysis the EEG signals over the left frontal, central, parietal, and occipital cortices were re-referenced to the right mastoid (F3-A2, C3-A2, P3-A2, and O1-A2). Visual and semiautomatic artifact removal was performed based on 2 frequency bands (0.75 to 4.5 Hz and 20 to 30 Hz; Huber et al. 2000). For the detection of slow waves, an automatic detection algorithm similar the one described in the work of Riedner and colleagues (2007) was applied. After band-pass filtering (Chebyshev Type 2 Filter: pass-band 0.5 and 4.0 Hz, stopband: <0.16 and >10 Hz) sleep slow waves during artifact-free NREM sleep epochs (i.e., QS at 2 months of age and stage 2, 3, and 4 of NREM sleep at higher age) were detected

as negative deflection of the EEG signal between 2 consecutive zero-crossings. The negative deflection was chosen due to the higher stability of the signal. However, it has been shown that peak-to-peak analysis shows similar results (Riedner et al., 2007). Only negative half-waves with a frequency between 0.5 and 2 Hz (of any amplitude) were considered for further analysis. From these detected half-waves, we determined the point in time of all zero-crossings and amplitudes (local minima of the signal). The ascending slope of slow waves was then defined as the amplitude divided by the time between the point in time of the local minimum and the subsequent zero-crossing (Fig. 1A). From these detected waves, the mean amplitude and slope of slow waves were calculated across the entire night. The incidence of slow waves was expressed as number of slow waves detected per hour of NREM sleep.

Next, we were interested in overnight changes of the slope of slow waves. Therefore the amplitude differences need to be controlled for. To compare the slope of slow waves with the same amplitude, we chose a similar approach as Bölsterli et al. (2011). The amplitudes were plotted against the slopes of all detected slow waves separately of the first hour (first 60 min of artifact-free NREM sleep) and last hour (last 60 min of artifact-free NREM sleep) of NREM sleep. We considered only waves with amplitudes included in the overlapping amplitude range for the first and the last hour of NREM sleep. Next, a linear regression between the amplitude and the slope for the first and last hour of NREM sleep was calculated (Fig. 1B). Based on the regression parameters (Slope(amplitude) = b + a(amplitude), b=intercept, a=slope), we calculated the slope at a fixed amplitude of 55 µV for the first and last hour. This procedure resulted in an approximate value for the slope of slow waves with amplitudes of 55 µV for the first and last hour of NREM sleep. We termed this slope “slope55.” An amplitude of 55 µV was chosen because all infants at all ages exhibited a large number of slow waves with this amplitude (see Fig. 3). However, similar results (i.e., an age-dependent increase and an overnight decrease in the slope) were obtained when choosing higher amplitudes (i.e., 75 µV or 100 µV) to account for age dependent differences in slow wave amplitude.

In the previous study with the same dataset, it was shown that the alternating pattern of low-delta activity is most prominent at the age of 6 months (Jenni et al., 2004) Thus, we investigated whether the alternating pattern is also seen in the slope55. Therefore the slope55 for the first 7 consecutive NREM episodes (for cycle criteria see; Jenni et al. 2004) in a subset of 6 infants at the age of 6 months were calculated. Only infants with a clear alternating pattern in low-delta activity during the first 7 consecutive NREM episodes were included in this analysis.

For an assessment of the propagation properties of slow waves we calculated the ratio between local and global slow waves during the first hour of NREM sleep. Because we were interested in global slow waves, all waves with high amplitudes (top 10%) were detected. Next, all waves occurring across all 4 channels within a time window of 100 ms were defined as global slow waves. It has been shown that slow waves need approximately 100 ms to sweep across the entire cortex (Massimini et al., 2004). All the other waves were defined as local waves. For this analysis one outlier (>> than 95 quantile of the group) in the 4-month-old age group had to be excluded, and one night of a 2-month-old subject because one of the 4 channels had too many artifacts.

Statistics

Changes of slow wave characteristics during the first 9 months were based on data of the conventional derivation C3-A2. Since the other derivations revealed similar results as C3-A2, only data from the latter are presented. For the analysis of topographical changes all channels from the left side (F3-A2, C3-A2, P3-A2, and O1-A2) were included. For the comparison of slow wave characteristics between different age groups, a linear mixed model ANOVA was calculated (random intercept: subject (to account for repeated measurements of the same person); main factor: age). For the analysis of overnight changes and regional differences, the additional fixed effects time (first and last hour of NREM sleep) or channel was added. In case of significance in the ANOVA, post hoc Student t-tests were used for pairwise comparisons within or between age groups. The significance level was set at 5%. All data are presented as mean ± standard errors (SE). All analyses were performed using the software package MATLAB (Math Works) or SPSS 16.0.

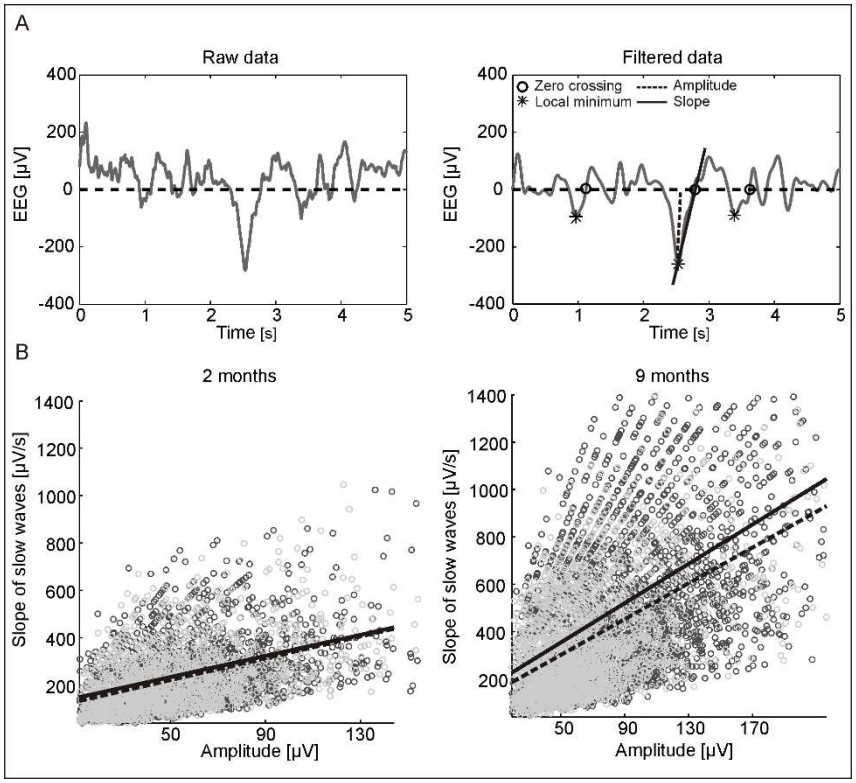

Fig. 1

(A) Schematic illustration of the slow wave detection method: 5 s of raw (left) and band-pass filtered (right) EEG signal of the C3-A2 derivation during NREM sleep. Right: Stars indicate detected slow waves. The slope was defined as the amplitude (local minimum of the signal, dotted line) divided by the time interval between the local minimum and the subsequent zero crossing (circles). (B) Example of detected slow waves for the derivation F3-A2: Scatter plots of all selected waves of the first (black) and last (grey) hour of NREM sleep of one subject at 2 (left) and 9 (right) months. For the first (continuous line) and last (dotted line) hour, a regression line was fitted to the data. Based on the regression parameters (slope and intercept), the slope at a fixed amplitude was determined.

Results

Because of electrocardiogram (ECG) artifacts in the reference channel A2, all recordings at 2 weeks after birth and all sessions of one subject (boy) were excluded from further analysis. Furthermore, at ages 6 and 9 months, 1 and 3 subjects, respectively, were excluded from analysis because sleep recordings were too short (total sleep time [TST] < 386 min). Thus, a total of 10 infants at 2 and 4 months, 9 at 6 months, and 7 subjects at 9 months (see Table 1) were analyzed.

Sleep Architecture and General Changes in Characteristics of Sleep Slow Waves during Infancy

Minor differences in sleep architecture between this current and the previous analysis (Jenni et al., 2004) were due to the inclusion of a different number of subjects (all recordings with a minimal amount of 386 min TST were included in the current analysis). Total sleep time (TST) increased with age (~ 22.3% from 2 months to 9 months). The amount of NREM sleep expressed as percentage of TST also showed an increase, whereas the amount of REM sleep decreased with age. Wake after sleep onset (WASO) and sleep efficiency (SE) did not differ across ages (Table 1).

	Age				
	2 mo	**4 mo**	**6 mo**	**9 mo**	**F_{age} (p)**
TRT [min]	636.9 ± 28.2	651.7 ± 31.2	691.5 ± 23.9	712.1 ± 26.4	1.5 (n.s.)
TST [min]	471.6 ± 13.0	513.3 ± 18.9	567.7 ± 26.2	576.6± 41.5	4.7 (0.01)
NREMS [%]	57.5 ± 1.7	62.3 ± 1.3	62.9 ± 0.8	63.8 ± 2.0	4.5 (0.01)
REMS [%]	42.5 ± 1.7	37.7 ± 1.3	37.2 ± 0.8	36.23 ± 2.0	4.5 (0.01)
IS [%]	5.1 ± 2.0	NA	NA	NA	NA
WASO [min]	62.5 ± 13.7	58.8 ± 12.4	52.4 ± 6.8	36.4 ± 8.5	0.8 (n.s.)
SE [%]	83.6 ± 1.9	86.0 ± 1.9	88.2 ± 1.2	90.8 ± 1.8	2.8 (n.s.)
n	10	10	9	7	

Table 1: Seep variables

Data are presented as mean ± SE. Between group differences were compared using a linear mixed model ANOVA with factor age. TRT: total recording time; TST: total sleep time; NREMS: non-rapid eye movement sleep (expressed as % of TST), REMS: rapid eye movement sleep (expressed as % of TST); IS: indeterminate sleep (expressed as % of TST), WASO: wake after sleep onset; SE: sleep efficiency (TST as % of TRT); n: number of subjects per age group. NA: not applicable, n.s.: not significant.

The main focus of this paper was the characterization of slow waves (i.e., negative half-waves) during NREM sleep in infants. In a first step, we calculated the average amplitude and slope of slow waves during NREM sleep for the entire night. Both, amplitude and slope increased with age from 50.9±1.4 μV and 250.8±8.3 μV/s at 2 months to 72.6±2.9 μV and 425.9±14.8 μV/s at 9 months, respectively (ANOVA $F_{age} > 18$, $P < 0.001$). The overall incidence of slow waves/h NREM sleep showed no age dependent changes during the first 9 months (i.e., 2150.7±100.5 slow waves/ h NREM sleep at 2 months, 2392.5±64.4 slow waves/ h NREM sleep at 9 months, ANOVA $F_{age} = 1.00$, $P > 0.4$).

Overnight Changes in Sleep Characteristics during Infancy

We were also examining whether slow wave characteristics change across the night, which may indicate homeostatic regulation. Thus, for each age group, the average amplitude, slope, and incidence of slow waves were calculated separately for the first and last hour of NREM sleep (Fig. 2). For the amplitude and slope of slow waves we found a consistent decrease from the first to the last hour of NREM sleep (ANOVA $F_{Time} > 47$, $P < 0.001$). In contrast, the number of detected slow waves/h NREM sleep increased slightly from the first to the last hour (ANOVA $F_{Time} = 6$, $P = 0.02$).

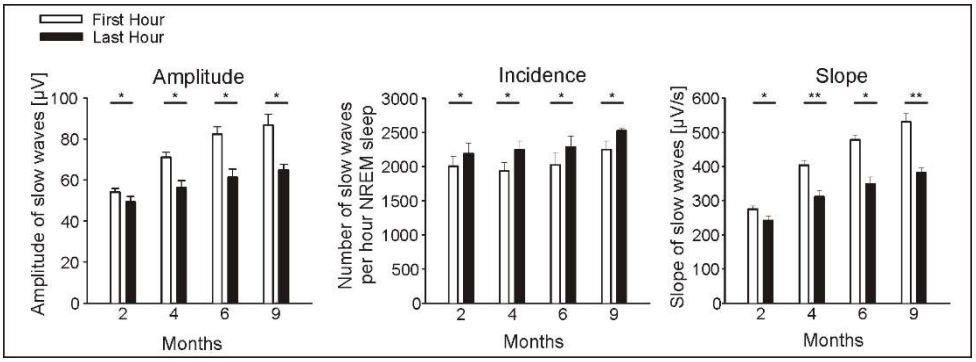

Fig. 2

Characteristics (amplitude, incidence, and slope) of slow waves for the first and last hour of NREM sleep (C3-A2): Bars indicate group means ± SE. Paired t-test were used to test differences within age groups from the first to last hour of NREM sleep (* $P < 0.05$, ** $P < 0.001$).

Next, we analyzed the slope of slow waves for the first and last hour of NREM sleep for all waves with any given amplitude to verify whether there are any amplitude specific changes. Therefore, we calculated the mean slope of slow waves of all waves within 10 µV amplitude bins (Fig. 3). The decrease in the slope of slow waves from the first to the last hour of NREM sleep was observed for the entire amplitude range at each age (ANOVA $F_{Time} > 73$, $P < 0.001$).

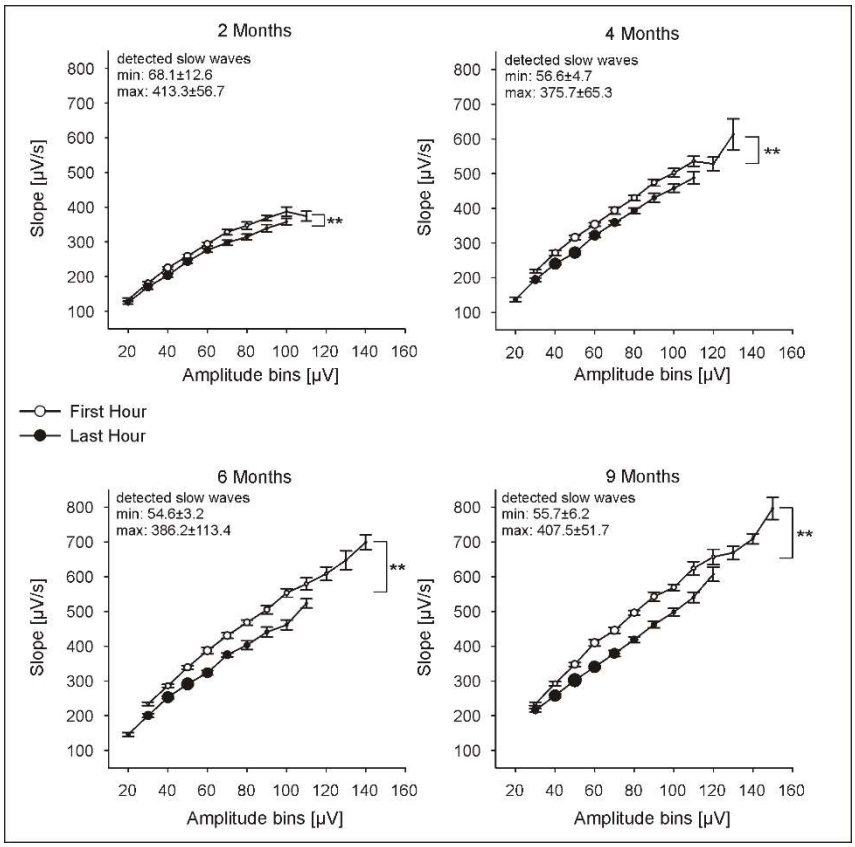

Fig. 3

Slope of sleep slow waves for consecutive 10 µV amplitude bins (numbers represent the upper limit) of the first and last hour of NREM sleep (C3-A2): group mean ± SE for both time points are shown. Only amplitude bins with at least 50 detected waves per subject are considered. Circle size represents the number of detected slow waves for each amplitude bin. Numbers in the upper left corner of each plot show the maximal and minimal number of detected slow waves across all subjects (mean ± SE). ANOVA factor time (first hour vs. last hour): ** P < 0.001, n ≥5).

To control for amplitude differences (for an unbiased assessment of the level of synchronization among population of cortical neurons) the slope of slow waves was calculated at a fixed amplitude of 55 µV (slope55) separately for the first and last hour of NREM sleep (for details see Methods; the amplitude of 55 µV was chosen as a representative amplitude because all infants at each age exhibited large amounts of slow waves at this amplitude, Fig. 3). The slope55 of the first hour of NREM sleep showed an overall increase with age (ANOVA F_{age} = 102.6, P < 0.001). When comparing the first to the last hour of sleep we found a consistent overnight decrease of the slope55 at each age (ANOVA F_{Time} = 140.6, P < 0.001, Fig 4). These results were not affected by our approach to control for amplitude differences, as done with the slope55, since the same results were obtained when we calculated the average slope of all detected slow waves with amplitudes between 50 and 60 µV (data not shown). The extent of the overnight decrease of the slope55 also increased with age (ANOVA F_{age} = 5.2, P = 0.005, Fig. 4 insert). This finding was independent of the increase in TST with age, because a similar result was found when equal amounts of sleep (only the first 386 min of sleep) were included (ANOVA F_{age} = 4.2, P = 0.01). Moreover, the steeper the slope during the first hour of NREM sleep, a larger overnight decrease was observed, independent of total sleep time (partial correlation, Rho = 0.7, P < 0.001).

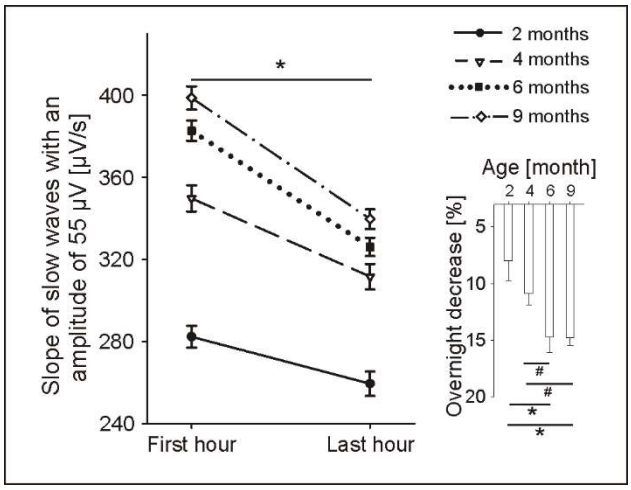

Fig. 4

Slope of slow waves with an amplitude of 55 µV (slope55) of the first and the last hour of NREM sleep (C3-A2): group means ± SE for both time points are presented (Paired t-test First hour vs. last hour: *P < 0.002). Insert: Overnight decrease in slope55 (in percentage of the first hour): Bars indicate group means ± SE (Paired t-test: * P < 0.05, # P < 0.06).

Time Course of the Slope55 and the Alternating Pattern at Age 6 Months

In a next step, we were interested whether the alternating pattern is also reflected in the slope55 at the age of 6 months when the alternating pattern was most prominent in low-delta activity. Interestingly, compared to low-delta activity we found a steady decrease of the slope55 across the first 7 consecutive NREM episodes in these infants (ANOVA $F_{episode} = 11.7$, $P < 0.001$, Fig. 5).

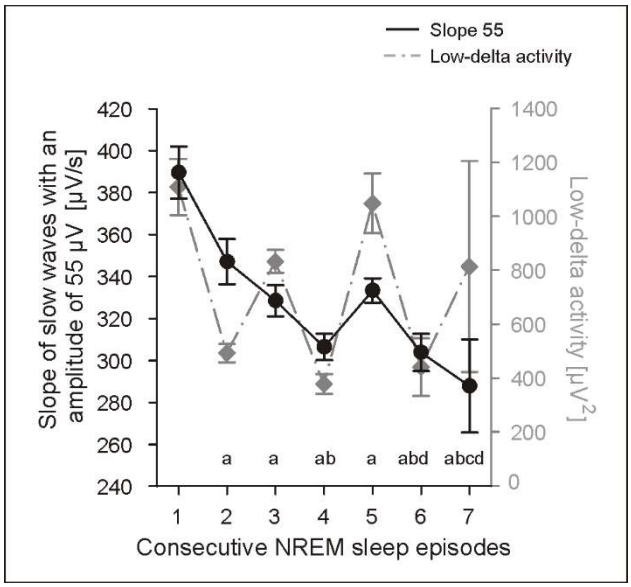

Fig. 5

Slope of slow waves with an amplitude of 55 µV (slope55) of the first 7 NREM sleep episodes at the age of 6 months for 6 infants: Group mean values ± SE. (a $P < 0.006$ vs. 1st NREM episode, b $P < 0.008$ vs. 2nd NREM episode, c $P < 0.008$ vs. 3rd NREM episode, and d $P < 0.05$ vs. 5th NREM episode). Low-delta activity (for details of the calculation see Jenni et al.11) is shown in gray (right ordinate) to illustrate the different time course.

Topographical Differences in the Slope of Slow Waves during Infancy

For the assessment of topographical differences, we calculated the slope55 of the first hour of NREM sleep for each channel (Fig. 6). In all derivations (frontal, central, parietal, and occipital), the slope55 increased with age (ANOVA $F_{age} = 182.8$, $P < 0.001$). Furthermore, the slope55 over the parietal, central, and frontal cortices were similar at each age, while the slope55 derived from the occipital derivation were much steeper than the other channels (ANOVA $F_{channel} = 69.4$, $P < 0.001$). In a preliminary analysis, we also calculated the slope55 of 2 children at the age of 2 years. We found a further increase of the slope55 in frontal (+19.5%), central (+18.1%), and parietal (+7.3%) derivations, but no increase in the occipital derivation (-2.5%)

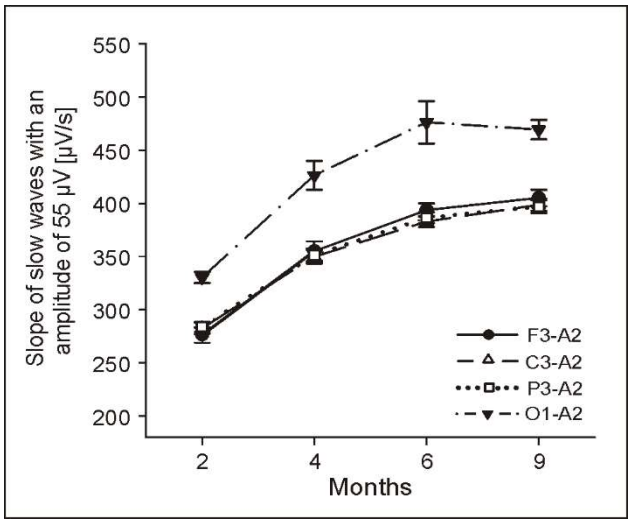

Fig. 6

Topographical differences in the slope of slow waves with an amplitude of 55 µV (slope55) of the first hour of NREM sleep: Values represent group means ± SE for the frontal, central, parietal, and occipital derivation (referenced to the right mastoid). Significant group effects were found for factor age and channel (F_{age}=182.84, P < 0.001; F_{chan} = 69.68, P < 0.001); 2 mo: n=10 (except for channel F3-A2 n=9), 4 mo: n = 10, 6 mo: n = 9, 9 mo: n = 7).

To assess the global nature of sleep slow waves as a marker for increased cortical connectivity the amount of global slow waves during the first hour of NREM sleep were calculated. We found that the majority of slow waves occurred locally (~ 94%). However, the amount of global slow waves increased with age (ANOVA F_{age} = 8.24, P = 0.001) from 3.81% at 2 months to 8.57% at 9 months (P < 0.001).

Discussion

We found a decrease in the amplitude and the slope of slow waves from the first to the last hour of NREM sleep in infants. When controlling for the amplitude difference we still observed an overnight decrease in the slope (slope55) at all ages. Our findings suggest that the level of synchronization among populations of cortical neurons, a cellular counterpart of the homeostatic regulation of sleep,14 decreases across the night in infants.

The electrophysiological origin of sleep slow waves is a changing pattern of neuronal silence (on-state) and neuronal activity (off-state) of cortical neurons (Steriade et al., 2001). If this slow oscillation is highly synchronized among large populations of cortical neurons, they become detectable as large amplitude slow waves in the surface EEG (Vyazovskiy et al., 2009). Measuring simultaneous multiunit activity and local field potential in rats, Vyazovskiy et al. showed that the synchronization of the transition between on- and off-states is reflected by the slope of slow waves in the surface EEG (Vyazovskiy et al., 2009). Thus, the faster populations of cortical neurons can synchronize their neuronal activity, the steeper the slopes of slow waves are. Such changes in the synchronization of the neuronal firing pattern reflect a cellular counterpart of the homeostatic regulation of sleep. Increasing sleep pressure leads to a high synchronization between on- and off-states in large populations of cortical neurons, associated with steep slow waves in the surface EEG. When sleep pressure dissipates with progression of sleep, this synchronization declines, resulting in shallower waves. Therefore, we used the slope of slow waves as an electrophysiological marker for the homeostatic regulation of sleep. Comparing slow waves with the same amplitude our results show that the slope decreased from the first to the last hour of NREM sleep in infants at the age of 2 to 9 months. Thus, our findings provide evidence for a homeostatic regulation of cortical neuronal activity already at the age of 2 months.

We note that such changes of the slope of slow waves with fixed amplitude are accompanied by a shift in frequency. More specifically, if the amplitude of a slow wave is fixed, an increase in the slope goes along with an increase in the frequency of this wave. When calculating the frequency55 (frequency of all waves with an amplitude of 55 μV), the results were similar, i.e., frequency55 showed an overnight decrease and an age-dependent increase.

However, the question remains why the level of neuronal synchrony would increase with the duration of wakefulness and decrease during sleep. The synaptic homeostasis hypothesis claims that an increase in the strength of cortico-cortical connectivity during wakefulness is followed by a decrease (downscaling) during subsequent sleep (Tononi and Cirelli, 2006). In fact, evidence is accumulating that net synaptic strength is tightly regulated over 24-h periods; it increases during wakefulness and decreases during sleep (Bushey et al., 2011; Gilestro et al., 2009; Vyazovskiy et al., 2008). Moreover, a computer model of the thalamocortical system provided evidence that an increase in synaptic strength would improve the synchronization of neuronal activity leading to steeper slow waves in the EEG (Esser et al., 2007). Kurth and coworkers showed that the reduction in synapse density during puberty is reflected in a decrease in the slope of slow waves (Kurth et al., 2010a) Further support for this notion is provided by our analysis of regional differences in the slope55. The slope55 of the first hour of NREM

sleep increased in all derivations (frontal, central, parietal, and occipital). However, the slope55 over the occipital cortex was the steepest at all ages. This time course of the slope55 is related to the time course of synaptogenesis of the human cerebral cortex (Huttenlocher, 1990). Synapse density experiences a vast increase during the first postnatal months, reaching a peak at around 7 months in the occipital cortex and about 3 years later in the auditory and prefrontal cortices (Huttenlocher and Dabholkar, 1997). In an explorative investigation, we also calculated the slope55 of 2 children at the age of 2 years. In this preliminary analysis, in line with the morphological data of Huttenlocher and Dabholkar (1997), we found a further increase of the slope55 in frontal, central, and parietal derivations, but no increase in the occipital derivation. Thus, the increase in synaptic strength, reflecting increased cortical connectivity during infancy could lead to an improved synchronization of neuronal activity, which would explain the observed rise of the slope55. Additional evidence for this hypothesis comes from the observation that the amount of global slow waves increases with age. Thus, a better connected network would facilitate the propagation of slow waves across the cortex and therefore lead to more global slow waves in the EEG. The extent of the overnight decrease of the slope55 also revealed an age-dependent increase. Thus, it seems that the increase in cortico-cortical connectivity goes along with increased downscaling across the night. In fact, the observation that the sloep55 of the first hour NREM sleep was positively correlated with the amount of the overnight decrease of the slope55, even when controlling for total sleep time supports this conclusion.

Previous studies concerning the development of a homeostatic regulation of sleep, relied either on the duration of slow wave sleep (Bes et al., 1991; Coons and Guilleminault, 1982; Sterman et al., 1977), or on the power in the delta frequency range (i.e., low-delta activity; Sterman et al. 1977; Jenni et al. 2004). However, it is still unknown when the homeostatic regulation of sleep develops in human life. For example, several studies reported that slow wave sleep becomes predominant in the first part of the night in infants starting at 2 months of age (Bes et al., 1991; Coons and Guilleminault, 1982). On the other hand, low-delta activity seems to alternate between consecutive NREM/ REM cycles in infants (Jenni et al., 2004) compared to the continuous decrease of SWA in adults (Achermann and Borbély, 2003). A previous study with the same dataset showed that the alternating pattern is most prominent at 6 months (Jenni et al., 2004). At this age no decrease in low-delta activity in the course of the night was observed. Interestingly, when examining the slope55 (of waves within the low-delta frequency range), we found a decrease from the first to the last hour of NREM sleep. The time course of the slope55 across consecutive sleep cycles showed the same pattern (Fig. 6). We used the same frequency range for the analysis of the characteristics of sleep slow waves as used in the previous studies (0.5-2 Hz; Riedner et al. 2007; Kurth et al. 2010; Bölsterli et al. 2011). Because the frequency range for the low-delta activity (0.75-1.75 Hz), for which the alternating pattern was described (Jenni et al., 2004), slightly differs, we repeated our analysis considering only waves between 0.75 and 1.75 Hz. We found a similar decline of the slope of slow waves across the night (Slope55 of NREM sleep episodes 1: 378.3±8.1 µV; 2: 351.2±6 µV; 3: 334.7±4.4 µV; 4: 319.4±2.8 µV; 5: 338.8±4.3 µV; 6: 317.2±7.4 µV; 7: 299.0±21.2 µV). These results point to a certain level of independency between the slope of slow waves and low-delta activity. This observation is of special interest because SWA predominantly depends on the amplitude (and incidence) of slow waves. We found an overnight decrease in the amplitude, but an increase in the

incidence of detected slow waves. Thus, it might be possible that the decrease in amplitude from the first to the last hour of NREM sleep is compensated for by the increase in the incidence of slow waves, resulting in equal levels of low-delta activity from the first to the last hour of NREM sleep. Support for this notion comes from a study by Bersagliere and Achermann (2010) reporting that EEG power of waves oscillating below 1 Hz did not show the homeostatic increase after sleep deprivation at the beginning of recovery sleep in adults, even though the amplitude and slope increased.

In summary, we showed that the slope of slow waves (i.e., negative half-waves) decreases overnight in human infants starting at age 2 months. These results suggest an early development of the homeostatic regulation of sleep at the level of neuronal synchronization. In contrast, low-delta activity does not reveal a decrease in the course of the night at this age (Jenni et al., 2004). A possible explanation for this discrepancy might be that power, as a function of slow waves amplitude and incidence, might not only reflect neuronal synchronization, but rather additional mechanisms, for example the duration of the underlying off-state (Vyazovskiy et al., 2009). Interestingly, Jenni and colleagues (2011) showed that theta activity instead of low-delta activity may reflect a marker of sleep homeostasis in infants. This observation is of interest in light of a recent multiunit recording study in awake rats showing evidence that the underlying cellular mechanism of theta activity and SWA are related (Vyazovskiy et al., 2011). The question remains whether the level of neuronal synchronization is also reflected in the slope of theta waves. The low sampling rate of our dataset did not permit to address this question. Another important limitation that needs to be considered is that our recordings were limited to the nocturnal sleep period. Unfortunately, for practical reasons it was not possible to perform 24-h recordings including also daytime sleep. However, it has been reported that already after the first 6 weeks of life, consolidated nocturnal sleep episodes occur (Jenni et al., 2006). Moreover, mean TST was 471.6±13.0 min in our youngest age group, which points to consolidated nocturnal sleep.

In conclusion, our analysis of the slope of sleep slow waves provides evidence for age dependent changes in the generation and regulation of sleep slow waves during early human development. Thus, the slope of sleep slow waves might serve as a useful tool to study aberrant levels of neuronal synchronization (e.g., epilepsy). In general, such a tool may also be used as an electrophysiological marker of cortical maturation during infancy.

4.2 Oscillatory theta events in the waking electroencephalogram of children resemble local aspects of sleep during wakefulness

Sara Fattinger[1,2,3], Salome Kurth[1,2,3], Maya Ringli[1,2,3], Oskar G. Jenni[1,2,3], Reto Huber[1,3,4]

[1] Children's Research Center, University Children's Hospital Zurich, Switzerland
[2] Child Development Center, University Children's Hospital Zurich, Switzerland
[3] Neuroscience Centre Zurich
[4] Department of Child and Adolescent Psychiatry and Psychotherapy, Psychiatric Hospital, University of Zürich, Switzerland

Financial support was provided by the Swiss National Science Foundation grants PP00A-114923 (R.H.), a research grant from the Zurich Centre for Integrative Human Physiology (R.H., O.G.J., P.A.) and a research grant from the University Research Priority Program of the University of Zurich (Clinical Research Priority Program Sleep and Health)

Own contribution: analysed the data, wrote and edited the manuscript

Submitted

Abstract

Vyazovskiy and colleagues (2011) found in rats' multi-unit recordings brief periods of silence (off-states) in local populations of cortical neurons during wakefulness. These periods of silence closely resembled the characteristic off-states during deep sleep. Moreover, increasing sleep need by sleep deprivation resulted in more frequent and more spatially spread (i.e. widespread) local off-states. Finally, when rats were subjected to a food-pellet reaching task, they found significantly more off-states prior to an unsuccessful compared to successful reaching attempt. While these animal experiments were based on intracranial recordings, we aimed to explore whether the human surface EEG may also provide evidence for such a local intrusion of sleep-like activity during wakefulness. Thus, we analysed high-density wake EEG recordings (128 electrodes) during a 4-min auditory attention task in the morning and evening in 12 healthy, normally developed children (age 10.5 ± 0.3 years, 3 females). Our results showed that (1) theta waves became more widespread in the evening and (2) the occurrence of widespread theta waves was associated with slower reaction times in the attention task. We conclude that theta waves recorded in children's surface EEG show properties comparable to the local, sleep-like off-states in multi-unit recordings of awake rats.

Keywords: theta activity, local sleep, single wave detection, high density EEG

Introduction

Sleep and wakefulness are clearly separable brain states, but are yet critically dependent on each other. The separation of the two states has not only been defined on the behavioural, but also on the electrophysiological level. Physiological deep sleep is associated with a slow oscillating pattern of brain activity and the absence of physical activity, while waking behaviour is associated with continuous brain activity (Steriade et al., 2001). The dependence on each other is best illustrated by the homeostatic regulation of sleep found in animals and humans. Sleep need grows with time spent awake and can only be dissipated during sleep (Borbély, 1982). Thus, on the one hand, increased sleep need due to insufficient sleep has significant behavioural consequences during next day's waking period. On the other hand, consolidated wakefulness is also important for the build-up of sleep need, which then ensures a good night sleep. For both aspects, the build-up of sleep need and its dissipation during sleep, established electrophysiological markers exist. The build-up of sleep need is reflected in electroencephalographic (EEG) theta activity (EEG power between 6-8 Hz) during wakefulness (Aeschbach et al., 1997b; Cajochen et al., 1995; Finelli et al., 2000; Hung et al., 2013). Slow wave activity (1-4.5 Hz) during sleep, on the other hand, reflects the level of sleep need at the beginning of the night and its consecutive recovery across the night (Achermann and Borbély, 2003). A key difference between the two states deep sleep and wakefulness is the change in properties of neurons. During deep sleep, the membrane potential of thalamo-cortical neurons become bistable (i.e., alternating between two different voltage level) due to a reduced drive of the arousal-promoting neuromodulators (Hill and Tononi, 2005). This bistability is seen at the individual neuronal level as an oscillation of the membrane potential between depolarization (on-state) and hyperpolarization (off-state; Steriade et al. 2001). As a result, neurons during deep sleep display an alternating activity pattern of periods (100-500 ms) with neuronal activity with a similar frequency as during wakefulness and periods of complete neuronal silence (100-300 ms; (Steriade et al., 1993b). Studying multi-unit activity in rats, Vyazovskiy et al. (2009) found that at the beginning of a sleep episode when sleep need is high, on-states are highly synchronized between individual units and associated with high amplitude slow waves. When sleep need dissipates in the course of sleep, the synchronization across units declines reflected in lower amplitude slow waves in the scalp EEG

However, most recent findings have challenged the concept of dichotomy of sleep and wakefulness on the neuronal level: the distinction of brain states as sleep or waking might only partially hold true. Evidence for wakefulness-like brain activity during sleep and local sleep during wakefulness has been presented in human and animal data (Nobili et al., 2012, 2011; Vyazovskiy et al., 2011). For example, intracranial recordings of brain activity in patients suffering from drug resistant epilepsy show clear signs of wakefulness-like activity typically observed over sensorimotor areas (Nobili et al., 2011). Using multi-unit recordings in rats, Vyazovskiy et al. (2011) described brief intermittent periods of silence (off-states) in local populations of cortical neurons during wakefulness. In contrast to the globally occurring slow oscillation pattern across almost all cortical neurons during slow wave sleep, the off-states during wakefulness seem to occur rather locally and last only a few ms. The shorter duration may explain why off-states during wakefulness were associated with theta (up to 6 Hz) rather than slow-wave activity in the waking EEG. Vyazovskiy et al. (2011) also explored the characteristics of these brief periods of

silence during prolonged periods of wakefulness and found that with increasing sleep need the local off-states became more frequent and involved larger cortical areas (i.e. became more widespread) which was associated with increased theta activity. Interestingly, in the human waking EEG, the increase in theta activity after sleep deprivation correlated with impaired performance (Cajochen et al., 1999; Makeig et al., 2000). Hence, the question arises whether local sleep (off-states) of cortical neurons during wakefulness which seem to reflect waking theta activity can account for the performance impairment. To address this question, Vyazovskiy et al., (2011) trained rats on a sugar pellet reaching task. The results showed significantly more off-states (300-800 ms) prior to an unsuccessful reaching attempt as compared to successful trials. These data indicate that local populations of cortical neurons that “fall asleep” may be responsible for the impaired performance following sleep deprivation.

All of the above mentioned findings have been drawn from intracranial recordings close to the neuron. In this study, we investigated whether the surface EEG likewise provides evidence for such local intrusion of one brain state into the other that is local sleep during wakefulness. Thus, we analysed high-density wake EEG recordings (128 electrodes) which provide a good spatial resolution and examined children because they generally show a pronounced increase in sleep need and a high signal to noise ratio.

Materials and Methods

Subjects and study design

The wake EEG of a subset of previously published sleep data (Kurth et al., 2010b), studied at the University Children's Hospital Zurich, Switzerland between 2008 and 2009 were analysed. All pre-pubertal children between 8 and 13 years participating in the former study were selected. EEG was recorded during wakefulness before (evening) and after sleep (morning) during an attention task (for details see below) in 16 children. Four, out of these 16 children were excluded from the analysis because of EEG artefacts, resulting in a total number of 12 participants included for analysis. All 12 children (3 females) were healthy and right-handed. Age ranged from 8.8 to 12.6 years (10.5 ± 0.3 years). Exclusion criteria for all subjects were personal or family history of psychopathology, severe brain injury, sleep disorders, chronic diseases, and current use of psychoactive agents or other medications. Participants were not allowed to travel across more than 1 time zone in the 4 months before the study (for further details see Kurth et al. 2010). Written informed consent was obtained from all children and their parents, after detailed explanation of the study design and aim. The study protocol was approved by the local ethics committee and was performed according to the Declaration of Helsinki.

Wake EEG Recording

High-density wake EEG (Electrical Geodesics Sensor Net for long-term monitoring, HydroGel, 128 channels, Electrical Geodesics, Eugene, OR, USA) was recorded for 4 minutes during an attention task in the evening right before going to bed and in the morning ~ 30 minutes after wake up. To ensure habitual levels of sleep need in the evening, subjects kept a regular sleep-wake cycle 7 days prior to the recording. Compliance with the schedule was verified by daily sleep diaries completed by the subjects or parents and the recording of activity by means of wrist actigraphy. No naps were allowed 24 hours before the recording day and all subjects were instructed to avoid alcohol and medication. Postpubertal females were recorded during the follicular phase to prevent the variation of EEG activity markers as a consequence of the menstrual cycle phase (Driver et al., 1996). Sleep was scheduled individually according to subject's reported habitual bed times. In the morning subjects were woken up in order to attend school. For the EEG recording the electrode net was adjusted to the vertex and the mastoids, and all electrodes were filled with electrolyte gel. Impedances were measured prior to EEG recordings in the evening and morning and kept below 50 kΩ.

Attention task

During the wake recording subjects performed an attention task based on an auditory oddball paradigm. Subjects were instructed to sit quietly in front of a screen and fix a white cross on a black background. Over a period of 4 min, participants listened to 300 stimuli (~80 dB) with an inter-stimuli interval of 0.8 s, whereof 90 % were standard tones (880 Hz) and 10% were deviant tones (988 Hz, presented in random order). Subjects were asked to respond to the deviant tones with a mouse click as quickly as possible. The task was programed with MATLAB (Math Works, psych toolbox). As performance measure, the reaction time to deviant tones was assessed. For performance data, four subjects in the morning and 2 subjects in the evening were excluded due to technical issues related to the simultaneous

acquisition of behavioural and EEG data. Thus, data of 6 subjects was available for both, the morning and evening.

Pre-processing of the wake EEG recording

EEG data were sampled at 1000 Hz (0.01 - 400 Hz), and recorded to the vertex (Cz). For offline analysis, the EEG signal was pre-processed similar to Hung et al. (2013). After passband filtering (0.1 - 48 Hz), poor-quality channels were identified by an automatic outlier detection based on amplitude threshold criteria, and were confirmed by visual inspection. Rejected channels were interpolated using spherical splines (NetStation, Electrical Geodesic Inc.). Next, data were transferred into Matlab and an Independent Component Analysis (ICA, using EEGLAB routines; Delorme and Makeig 2004) was performed to remove ocular, muscular, and electrocardiographic artefacts. Only ICA components with specific activity patterns for ocular, muscular and electrocardiographic artefacts were removed (Hulse et al. 2011). On average 7.9 ± 0.7 components were removed. In a last step, data were retransferred into NetStation to remove residual slow ocular artefacts (using Ocular Artefact Removal tool of NetStation). Further analyses were performed in Matlab. In addition to the above described pre-processing, a semiautomatic visual artefact rejection was performed based on power values in two frequency bands (0.75 - 4.5 Hz and 20 - 30 Hz). To do so the signal was down sampled to 128 Hz, and all 4-s epochs and channels containing artefacts were removed (Huber et al., 2000). On average 23.5 ± 1.9 epochs were removed in the evening and 24.1 ± 1.9 epochs in the morning, leading to ~2.7min of artefact free EEG recording for further analysis (evening: 2.7 ± 0.2 min; morning 2.7 ± 0.1 min). For each subject, only artefact-free channels in the morning and evening were included. For topographical analysis of theta power, the remaining data of each subject were re-referenced to an average value across all 109 channels above the ears that were not previously excluded. Theta power was calculated for each channel between 6 and 8 Hz (FFT routine, Hamming window, 4 s epochs, resolution of 0.25 Hz).

Detection of theta events

Theta event detection was based on the average referenced EEG signal which was passband filtered in the theta frequency range (Chebyshev Type 2 Filter: pass-band 5 and 9 Hz, stopband: below 4 and above 12 Hz). An automated detection algorithm adapted from Massimini et al. (2004) was designed: In a first step, the signal was averaged within 4 large non-overlapping areas of the scalp (for illustration of the four areas see Figure 1 b, in Massimini et al., 2004). Next, two times the standard deviation of absolute amplitudes were computed for each area and averaged across the four areas. This value was considered as detection threshold. Finally, the event detection algorithm was applied to each channel separately using the following criteria: (1) time between negative zero crossing and a subsequent positive zero crossing within 0.06 - 0.083 s (corresponding to 6 - 8 Hz), (2): negative-to-positive peak-to-peak amplitude > 2 times detection threshold, (3): negative peak > detection threshold (an example of the detection algorithm is shown in Figure 1 C).

In a next step, for each theta event the cluster size (*i.e.*, number of channels involved in the theta event) was calculated. We assumed that theta waves travel across the cortex comparable to slow waves

(Massimini et al., 2004). It is estimated that slow waves sweep across the entire cortex in approximately 100 ms (Massimini et al., 2004). Therefore, cluster sizes were calculated considering 5 different detection-windows (20 ms – 100 ms, in 20ms bins): for each detected theta event the number of neighbouring channels involved the given detection window was counted. For each event we assessed the following parameter: the number of channels involved in the maximal cluster size, the channel location and the respective amplitude (average across the channels within cluster).

Alignment of performance and EEG data

The reaction times to each deviant tone were considered as behavioural variables to quantify performance. All mouse clicks occurring more than 700 ms after the presentation of the deviant tone were defined as missed clicks. To investigate the relationship between the occurrence of theta events and reaction times, a time window was defined starting 900 ms prior to the stimulus time point (deviant tone) and ending 100 ms after the stimulus time point. Then the automated theta event detection algorithm and the calculation of cluster sizes (as described above) were repeated for this time frame.

Statistics

Inferential statistics were computed using linear mixed effects models, because they account for covariance between related data samples in repeated measures designs (Gueorguieva and Krystal, 2004). The mixed effects models were fitted using restricted maximum likelihood estimation (REML) and an ante-dependent first order covariance matrix. In advance, the model fit was verified depending on model fit indices (Akaike Information Criterion and Schwarz Bayesian Criterion). F tests were used to estimate the influence of each fixed effects on the model. "*Subjects"* was considered as a random effect with random intercepts. For the comparison of cluster sizes between morning and evening for different detection windows a mixed effects model was performed with repeated fixed effect of "*time"* and "*detection window"*. For comparison of the amplitude of different cluster sizes between morning and evening a mixed effects model with repeated fixed effect "*time"* and "*cluster sizes"* was performed. For all intra-individual comparisons, two-sided paired Student's T-Tests were used. For exceptional comparisons with sample size of 8 or smaller, Wilcoxon signed rank test (intra-individual) or a Wilcoxon rank sum test (inter-individual) were used (indicated in the text). Significance was set at the 5% level. All analyses were performed with the software package MATLAB (Math Works, Version 14a) or SPSS (Version 22.0, SPSS Inc. Chicago, US). Data are presented as mean ± SEM.

Results

Pronounced increase of theta activity from morning to evening in children

Independent of the time of day (i.e., morning or evening), the amplitude of the wake EEG is much higher in children compared to adults (for an illustrative example see Figure 1). Thus children's EEG provides a much better signal to noise ratio, which facilitates the reliable detection of single theta events in the wake EEG. Moreover we observed a pronounced increase of theta activity form morning to evening by 44.8 ± 5.6 % in our participants, indicating that sleep need is accumulating fast in children. In the following steps, we established a method characterizing theta events in the waking EEG.

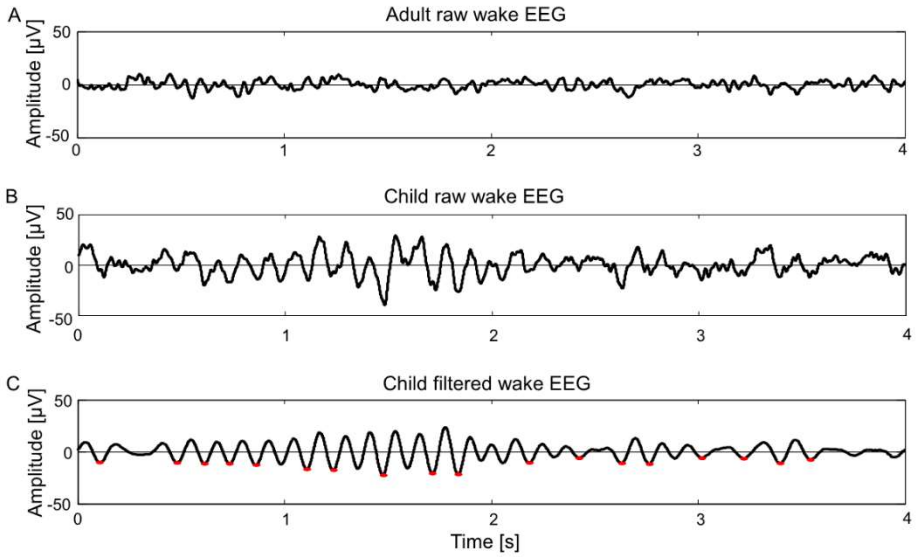

Figure 1

Representative examples of amplitude differences in wake EEG of a child and an adult. (A) Four seconds of the unfiltered wake EEG of an adult in the evening (male subject, 19.4 years old, from the data set presented in Kurth et al. 2010). (B) Four seconds of the unfiltered wake EEG of a child in the evening (male subject, 12.6 years old). Note, the amplitude of the wake EEG is much higher in children compared to adults. (C). Example of the theta event detection algorithm: the same 4 seconds EEG as in B are displayed, after filtering (Chebyshev Type 2 Filter: pass-band 5 and 9 Hz, stopband: below 4 and above 12 Hz). Grey asterisks mark the theta events detected by the algorithm.

Theta event detection and cluster size of theta events in the morning and evening

In a first step, we investigated whether theta events in the wake EEG are more global in the evening compared to the morning. Thus, single theta events and their cluster sizes (i.e., number of channels involved in a theta event) were detected. Similarly to the travelling of slow waves across the cortex (Massimini et al., 2004), we assumed that theta events are initiated at any site and spread across the cortex. As expected, a close relationship was found between cluster size and length of the detection window: the longer the detection window the larger the cluster size of theta events ($F_{\text{detection-window}} = 546.9$, $p < 0.001$, $n = 12$, Figure 2 A). Yet, independent of the considered detection-window, the cluster size of theta events was similar in the morning and evening ($F_{\text{time}} = 0.01$ $p = 0.93$; $n = 12$, Figure 2A). Moreover a similar number of theta events were detected in the morning and evening (mean across all

detection-windows evening: 2499 ± 137.75; morning: 2549 ± 143.5, p = 0.75, n = 12). The similarity in these parameters between morning and evening is likely a result of matching the amplitude detection threshold to each EEG recording (i.e., in the morning a lower amplitude threshold was needed to detect theta events compared to the evening: detection threshold morning: 3.34 ± 0.17 µV; evening: 3.92 ± 0.26 µV; p < 0.001, n = 12, for further details see methods). Because no differences in cluster sizes were found between morning and evening, all cluster sizes for each detection window were pooled across time points. Differences in the number of channels involved in a cluster across the different detection windows were computed (Figure 2 B). As shown in Figure 2 B, the increased number of channels involved in a cluster with increasing detection windows seems to level off after 60 ms. Thus, for further analysis we focused on a detection-window of 60 ms. The analysis considering detection-windows up to 100 ms are shown in the supplementary information.

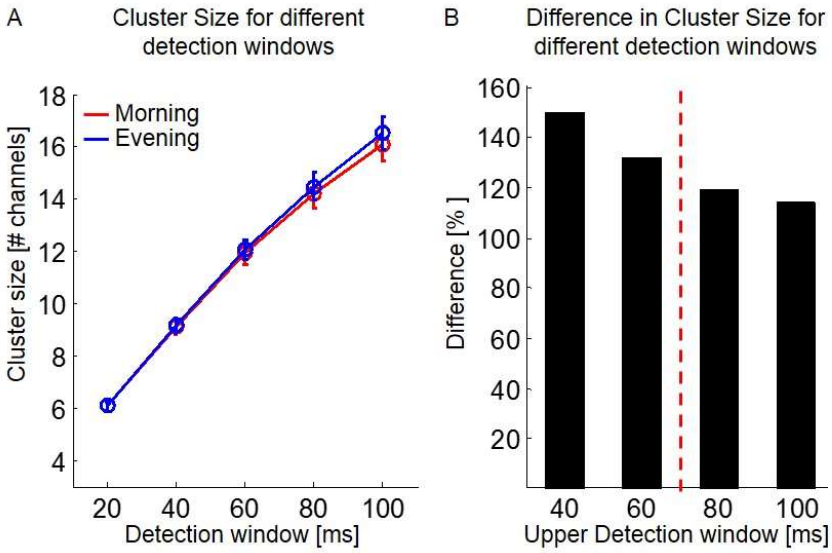

Figure 2

Relationship between detection-window size and cluster size. (A) Mean cluster size of theta events for different detection windows, based on an amplitude detection threshold matched to each EEG recording (mean ± SEM of cluster sizes for each detection window in the morning and evening are shown). The larger the detection window, the more channels are involved in the cluster sizes (linear mixed effect model: $F_{detection\text{-}window}$ =546.92, p<0.001, n=12). No difference in cluster size of theta events was found between morning and evening (linear mixed effect model: F_{time}=0.01, p=0.93 n=12). (B) The difference (percentage) of mean cluster sizes (*i.e.*, the number of channels involved in a cluster) between different detection windows. Morning and evening are pooled. X-axis represents upper detection window (*i.e.*, 40 = cluster size $_{Detection\ window\ 40}$/cluster size $_{Detection\ window\ 20}$*100). A detection window of 60ms (indicated by the grey dotted line) was selected for analysis.

The cortical involvement of theta events increases from morning to evening

Next, we elaborated the relationship between cluster size and theta wave amplitude. Independent of the time of day, a higher amplitude was associated with a larger cluster size ($F_{cluster\ Size}$ =32.61, p < 0.001, n = 12; detection window 60 ms; Figure 3A). For any cluster size, a higher amplitude was generated in the evening compared to the morning (F_{time} =207.1, p < 0.001, n = 12; Figure 3A; similar results were

obtained with a detection window of 100 ms, Figure S1). This result indicates that in the evening smaller clusters are needed to generate the same theta wave amplitude as compared to the morning. In a next step, we aimed at further investigating the cortical involvement of theta waves at the two time points by calculating the number of widespread theta events. Our data analysis showed that for a definition of a "widespread theta event" the cluster size as well as the amplitude of the wave have to be taken into account. Thus, to provide an unbiased view on the definition of a cluster of electrodes involved in a widespread event we performed two specific analyses.

1) Definition of cluster size threshold for widespread theta events

We pooled data across the two time points and divided all cluster sizes into 5-percentile bins (Figure 3B). Next, the difference in the number of channels involved in a cluster between each 5-percentile bin was computed (Figure 3C). For each increasing 5-percentile step, the cluster consisted of one or maximally two additional channels. However, exceeding the 85th percentile, the number of channels involved in a cluster increased stronger. We therefore defined the cluster size at the 85th percentile as the cutoff defining widespread theta events. This cutoff corresponds to a cluster of 20 channels. In other words, a minimum of 20 channels involved in a theta event accounted for a "widespread theta event".

2) Definition of amplitude threshold for widespread theta events

When defining an amplitude threshold for widespread theta events, it needs to be considered that a cluster of 20 channels generates a smaller amplitude theta wave in the morning compared to the evening (see above). Thus, to ensure that the definition of widespread theta events is not biased towards the evening, this amplitude difference was taken into account. Mean amplitude, which was generated by a cluster size of 20 channels, was calculated for the morning session in each subject and this value was considered as amplitude threshold for the morning and evening sessions.

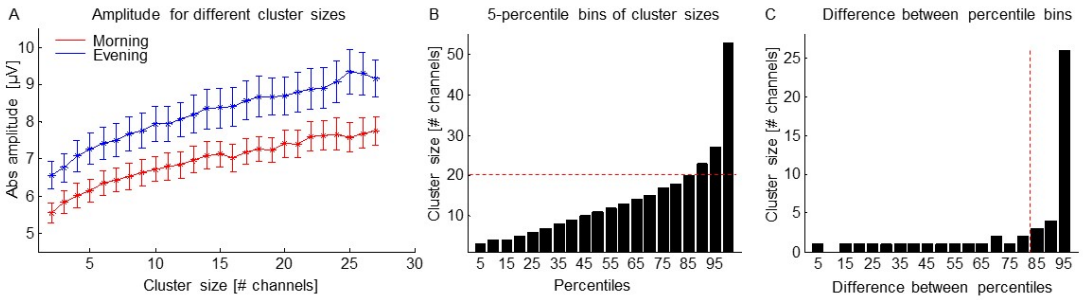

Figure 3

Definition of widespread theta events based on a detection window of 60ms. (A) Relationship between amplitude and cluster size in the morning and evening (mean ± SEM of the amplitude for each cluster size are presented for morning and evening. For each subject, mean amplitude was calculated when at least 5 theta events for the given cluster size were detected). Larger amplitudes were associated with increased cluster sizes (linear mixed effect model $F_{cluster\ size}$=32.61, p<0.001, n=12). For any cluster size a larger amplitude was detected in the evening compared to morning (linear mixed effect model: F_{time}=207.01, p<0.001, n=12). (B) 5-percentile bins of cluster sizes (morning and evening pooled). The 85th percentile corresponds to a cluster size of 20 channels (grey dotted line). (C) Difference in the number of channels involved in a cluster size between each 5-percentile bin from Figure B. Numbers on the x-axis indicate the lower bin (*i.e.*, 5 corresponds to the difference between the 5th and the 10th bin). Because the number of channels involved in the cluster size are increasing from the 85th percentile, the 85th percentile (corresponding to a cluster size of 20 channels, see B) was used for cluster size cutoff definition for widespread theta events.

Widespread theta events in the morning and evening

Next, we applied both thresholds (amplitude and cluster size) to morning and evening sessions. The results show that the mean cluster size of widespread theta events did not differ between morning and evening (morning: 26.0 ± 0.2 channels, evening: 25.9 ± 0.2 channels, p = 0.2, n = 12). Moreover, in the morning as well as in the evening, only a minority (~ 10 %) of all theta events were “widespread” (Figure 4). We finally calculated the percentage of widespread theta events for both time points and found a significant increase from morning to evening. Widespread theta events increased in each subject by an average of 64.3 ± 7.1 % (p < 0.001, n = 12, Figure 4A). Moreover, this increase appeared “globally”, as detections occurred in a large proportion of channels (Figure 4B), and was most pronounced across central and frontal areas. To exclude the possibility of a selection bias related to the detection window, we performed the same analysis for a detection window of 100 ms which confirmed the finding (percentage of widespread theta events morning: 9.7 ± 1.0 %, evening: 16.7 ± 1.7 %, p < 0.001, n = 12, with a cluster size threshold of 24 channels, see Figure S1).

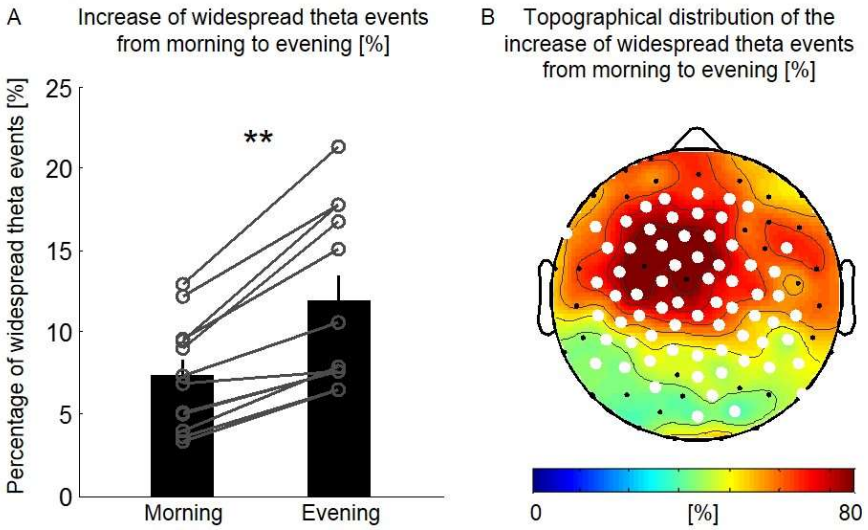

Figure 4

Increase of widespread theta events form morning to evening. (A) Percentage of widespread theta events in the morning and evening (bars represent mean ± SEM, circles represent individual subjects). Percentage of widespread theta events increase in all subject from morning to evening ($p<0.001$, paired student T-test, n=12). (B) Topographical distribution of widespread theta change from morning to evening (evening-morning/morning*100). Percentage of widespread theta events increase globally from morning to evening, which is most pronounced over central and frontal areas (white dotes $p<0.05$, paired student T-test, n=12).

Widespread theta events are associated with slower reaction times

Then we aimed at exploring whether widespread theta events are associated with impaired performance. Reaction times were similar between morning and evening (morning: 320.7 ± 25.9 ms; evening: 323.6 ± 19.3 ms, Wilcoxon rank sum test: $p = 0.8286$, $n_{morning} = 8$; $n_{evening} = 10$; Wilcoxon signed rank test: $p = 0.69$, $n = 6$). Therefore, for this analysis, the data from morning and evening were pooled. Theta events were detected within a time window starting 900 ms prior and ending 100 ms after the deviant tone. Within each subject all reaction times were grouped according to the following criteria: all reaction times associated with at least one widespread theta event were defined as “reaction times with widespread theta” and all reaction times in which no widespread theta event were detected were defined as “reaction time without widespread theta”. The number of reactions did not differ between the two groups (number without widespread theta: 11.0 ± 1.0; with widespread theta: 12.3 ± 1.0, $p = 0.52$). However, reaction times associated with widespread theta event were on average 12.88 ± 5.49 ms slower compared to reaction times without widespread theta events ($p = 0.036$, $n = 18$; Figure 5A; see Figure S2 for individual data).

Regional association between widespread theta events and impaired performance

Finally, we investigated whether this association between widespread theta events and slower reaction times was related to specific topographical areas. Theta events were grouped into fast reaction time (defined as the fastest 40% of reaction times) and slow reaction time groups (the slowest 40% reaction times, including the missed trials). For each group, the frequency of channels involved in a widespread

theta event was calculated. We found a cluster of 10 channels located over premotor cortex (Brodmann area 6, 5 channels), parietal cortex (Brodmann area 7, 3 channels) and somatosensory cortex (Brondmann area 5 & 3; 2 channels), which were more frequently involved in a widespread theta event in the slow reaction time compared to the fast reaction time group (slow reaction time group, 0.48±0.08; fast reaction time group, 0.31±0.06; p=0.01; Figure 5B).

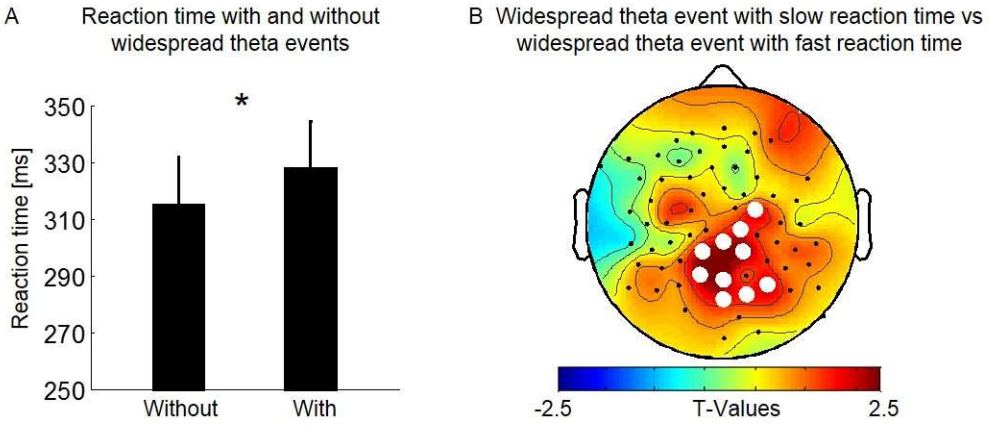

Figure 5

Relationship between widespread theta events and reaction time, data from morning and evening pooled. (A) Mean reaction time with no widespread theta event (= Without) and with at least one widespread theta event (= With). Reaction time associated with widespread theta are slower compared to reaction time without widespread theta events (mean ± SEM, p=0.036, paired student T-test, n=18). (B) Topographical comparison of widespread theta events and reaction time. All reaction times were split into two groups: the fast reaction time (defined as the fastest 40% reaction times) and the slow reaction time group (the slowest 40% reaction times, including the missed stimuli). For each group it was evaluated how often a channel was involved on average in a widespread theta event. A cluster of 10 channels (white dotes) was found, which were significantly more involved in widespread theta events in the slow reaction time compared to the fast reaction time group (white dotes p<0.05, paired student T-test, n=18).

Discussion

We report a ~45% increase of waking theta activity in the evening compared to the morning. In order to explore the underlying mechanisms of these changes in theta activity we ran single theta events detection and compared theta properties between morning and evening. Results show that widespread theta events are more likely occurring in the evening compared to the morning. Furthermore, widespread theta events were associated with slower reaction times in a simple auditory attention task. Thus, single theta events recorded in the human scalp EEG during wakefulness show properties comparable to the local off-states in multi-unit recordings of rats (Vyazovskiy et al., 2011). We propose that widespread theta events reflect local sleep during wakefulness.

In a first step, we discuss the evidence supporting our proposal that widespread theta events reflect local sleep. We would like to stress that only cellular recordings are capable to visualize sleep-like neuronal activity. Using cellular recordings, Vyazovskiy et al. (2011) showed the existence of local off-states during wakefulness in the rat. These off-states seemed to closely resemble the off-states occurring during sleep slow oscillations. Three main points support this conclusion: 1) local off-states during wakefulness become more global, and thus more sleep like, with increasing sleep need, 2) local off-states in unit activity are associated with increased theta activity in the surface EEG, our gold standard marker of sleep need during wakefulness, and 3) global off-states are related to performance impairments, as expected from sleep like activity. Our analysis revealed similar results for all three points.

The majority of theta events (~ 90 %) occur in locally restricted areas (8.7 ± 0.2 channels, morning and evening). Nevertheless, in every child, the percentage of widespread theta events was increased in the evening compared to the morning, indicating that the likelihood of widespread theta events increased with time spent awake. A change in an electrophysiological feature as a function of prior wake duration is typically described for SWA during NREM sleep (Borbély, 1982). Because numerous studies report a wake duration dependent increase of SWA (Achermann and Borbély, 2003; Borbely et al., 1984; Tobler and Borbely, 1986; Vyazovskiy et al., 2007a), SWA is considered the gold standard electrophysiological marker of homeostatic sleep regulation. Also the waking EEG informs about the homeostatic regulation of sleep: Several studies in adults revealed a pronounced increase of theta activity in the course of sleep deprivation (Torsvall and Akerstedt 1987; Cajochen et al. 1995; Aeschbach et al. 1997; Hung et al. 2013). Moreover, the increase in theta activity was related to the increase of SWA during initial recovery sleep (Cajochen et al. 1995; Aeschbach et al. 1997; Hung et al. 2013; Finelli et al. 2000). Furthermore, theta activity parallels subjective sleepiness and thus represents an objective measure of sleep need during wakefulness (Aeschbach et al., 1999; Akerstedt and Gillberg, 1990; Cajochen et al., 2001; Dumont et al., 1999; Strijkstra et al., 2003).

The second point supporting local sleep during wakefulness is the increase in theta activity with increasing sleep need (Vyazovskiy et al., 2011). In our sample, we observed a ~45% increase of theta activity in the evening compared to the morning. In contrast, theta activity in adults is only marginally increased in the evening compared to the morning (Cajochen et al., 2002; Finelli et al., 2000). This might

be due to a faster build-up of homeostatic sleep pressure during the day in children (Carskadon et al., 2004; Hagenauer et al., 2009; Jenni et al., 2005). Investigating neuronal activity uncovered an interesting feature of scalp theta activity. During wakefulness, cortical neurons are tonically depolarized and fire irregularly giving rise to an EEG pattern displaying low-amplitudes and fast-activities (Vyazovskiy et al., 2009). In contrast, during NREM sleep virtually all cortical neurons become bistable alternating between an active on- and a silent off-state (Steriade et al., 2001) leading to the prominent slow waves in the EEG signal (Vyazovskiy et al., 2009). When carefully aligning EEG theta waves during wakefulness with neuronal activity, subsets of cortical neurons showed brief intermitted periods of neuronal silence, even though the global EEG and behaviour show typical waking activity. These off periods were essentially indistinguishable from the off-states underlying sleep slow waves. Thus, theta waves measured on the surface seem to be associated with the occurrence of local, sleep like off-states on the neuronal level (Vyazovskiy et al., 2011).

Unquestionably, micro sleep, the intrusion of brief periods of electrophysiologically classified sleep into wakefulness results in behavioural deterioration with potentially dramatic consequences, for instance when driving (Lim and Dinges, 2008; Torsvall and Akerstedt, 1987). Thus, a key question is whether short local off-states during wakefulness are also associated with behavioural consequences. Indeed, Vyazovskiy et al. (2011) have shown that off-states during wakefulness result in impaired performance. When rats were performing a sugar pellet reaching task, the number of missed trials increased under high sleep need condition. Most importantly, missed trials were associated with local off periods in the primary motor cortex (Vyazovskiy et al., 2011). Our findings are in line with these observations. We show that preceding occurrence of more widespread theta events is related to a worse performance. More specifically, reaction times associated with widespread theta were longer compared to reaction times without widespread theta. Moreover, this relationship between reaction time and widespread theta was most pronounced over brain regions associated with motor execution (Figure 5B). Notably, overall reaction times were within a normal range (mean reaction time with widespread theta: 328.1±16.1ms; without widespread theta: 315.2±16.5ms). In addition, on average only 8 missed clicks (5 in the evening and 3 in the morning) were detected. Alternative tasks, like the task-switching paradigm, which is highly susceptible to sleep restriction (Couyoumdjian et al., 2010), may be more sensitive to assess worsening of performance due to the occurrence of local sleep. These initial findings, however, supported the suggestion that the occurrence of local sleep, measured as widespread theta events on the scalp surface of the awake brain, seem to be related to some cognitive impairments.

How neurons respond to incoming stimuli may depend on whether neurons are in an on- or off-period, which in turn may affect cortical functioning (Crochet and Petersen, 2006; Vyazovskiy et al., 2012). Strikingly, overall performance in sleep deprived subjects is characterized by fluctuating performance levels (i.e. alternating between normal and very bad performance; Doran et al. 2001; Zhou et al. 2011). The stochastic, all-or-none occurrence of off-periods in cortical neurons could indeed account for such fluctuations. Thus, the well-known negative consequences on performance when staying awake too long (Dijk et al., 1992; Van Dongen et al., 2003) may relate to the occurrence of local off-states during wakefulness. The correlation between increased theta activity in the waking EEG and performance

decrease in different studies may support this conclusion (Cajochen et al., 1999; Makeig et al., 2000). Unfortunately, our data does not allow to establish a direct relationship between theta activity and performance changes because of too short inter-stimulus intervals.

The last part discusses possible mechanisms underlying the increase in local sleep under conditions of high sleep need. Computer simulations of sleep slow waves show that amplitude and slope of slow waves are directly related to the number of neurons involved in on- and off-states. The more neurons enter an on- or off-state near synchronously, the higher the amplitude (Esser et al., 2007; Olcese et al., 2010). This is in line with our observation that higher amplitude theta waves are associated with increased cluster size, independent of the time of the day. If theta waves in the wake EEG underlie similar mechanism as slow waves during sleep, this result might indicate that higher amplitude theta waves might be generated by a larger subpopulation of cortical neurons simultaneously entering an off-state. Interestingly, theta events of the same cluster size (i.e., number of channels involved in a theta event) generated higher amplitudes in the evening compared to the morning. Computer models imply that synchrony within a neuronal network is determined by the number, strength and distribution of synaptic connections (Esser et al., 2007; Olcese et al., 2010). Thus, the larger amplitude in the evening compared to the morning may reflect increased synaptic strength. In line with the synaptic homeostasis hypothesis (Tononi and Cirelli, 2014), higher synaptic strength in the evening facilitates synchronized neuronal activity which in turn results in higher amplitudes. Alternatively, a decrease in arousal-promoting neuromodulators after falling asleep may facilitate a bistable state of the membrane potential as being the case when falling asleep (Hill and Tononi, 2005). To what extent changes in the milieu of neuromodulators contribute to the off-stages during wakefulness needs to be further investigated. The fact that theta events during wakefulness were rather local (i.e., also widespread theta events involved only ~26 channels), might indicate that the globally active neuromodulatory system during wakefulness prevents the emergence of global bistability.

In conclusion, assuming that short off-states of cortical neurons give rise to theta activity in the waking EEG, our findings suggest that in the evening, when the need for sleep is high, large subsets of cortical neurons become briefly bistable, and appear as widespread theta events in the waking EEG. According to the synaptic homeostasis hypothesis (Tononi and Cirelli 2014) increasing synaptic strength due to learning processes during wakefulness, has various costs at the cellular and system level, such as higher energy consumption, increased cellular stress, and associated changes on supporting cells (i.e. glial cells). This accumulating costs during wakefulness seems to drive single neurons towards off-states, allowing restoring cellular maintenance (Vyazovskiy and Harris, 2013). However, it remains speculative whether local off-states during wakefulness in humans fulfil restorative functions.

Supplementary material

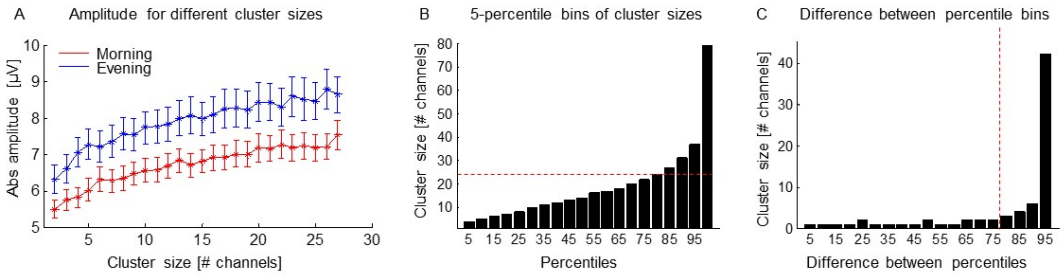

Figure S1

Definition of widespread theta events based on a detection window of 100ms. (A) Relationship between amplitude and cluster size in the morning and evening (mean ± SEM of the amplitude for each cluster size are presented for morning and evening. For each subject, mean amplitude was calculated when at least 5 theta events for the given cluster size were detected). Larger amplitudes were associated with increased cluster sizes (linear mixed effect model $F_{cluster\ size}$=43.2, p<0.001, n=12). For any cluster size a larger amplitude was detected in the evening compared to morning (linear mixed effect model: F_{time}=71.52, p<0.001, n=12). (B) 5-percentile bins of cluster sizes (morning and evening pooled). The 80th percentile corresponds to a cluster size of 24 channels (grey dotted line). (C) Difference in the number of channels involved in a cluster size between each 5-percentile bin from Figure B. Numbers on the x-axis indicate the lower bin (*i.e.*, 5 corresponds to the difference between the 5th and the 10th bin). Because the number of channels involved in the cluster size are increasing from the 80th percentile, the 80th percentile (corresponding to a cluster size of 24 channels, see B) was used for cluster size cutoff definition for widespread theta events.

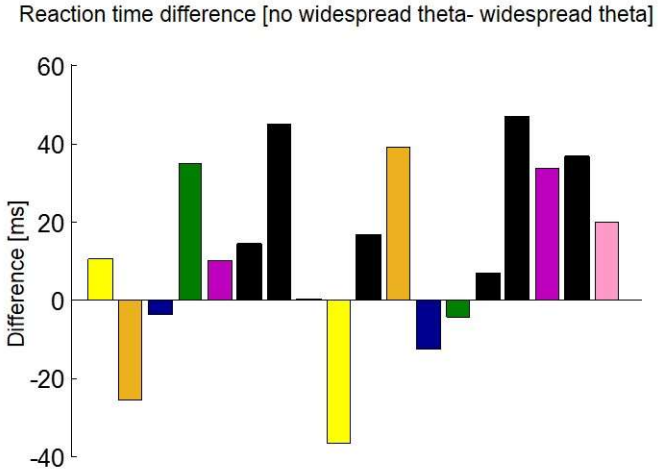

Figure S2

Difference in reaction time associated with widespread theta (=at least one widespread theta event) and reaction time without widespread theta for each intra-individual comparison. Since data of the morning and evening were pooled some subjects were considered twice (same colour=same subject, the first 8 comparisons correspond to the morning session). Note, the slower reaction times for stimuli associated with widespread theta seems not to be driven by the subjects which were included twice.

4.3 Electroencephalogram approximate entropy influenced by both age and sleep

Gerick M. H. Lee[1,2], Sara Fattinger[2], Anne-Laure Mouthon[2], Quentin Noirhomme[3] and Reto Huber[2]

[1] Institute of Neuroinformatics, University of Zurich and ETH Zurich, Zurich, Switzerland

[2] University Children's Hospital Zurich, Zurich, Switzerland

[3] Coma Science Group, Cyclotron Research Centre and Neurology Department, University and University Hospital of Li `ege, Li `ege, Belgium

Financial support was provided by Swiss National Science Foundation grant PP00P3-135438 to Reto Huber.

Own contribution: pre-processed the data, edited the manuscript

Published in
Frontiers in Neuroinformatics 2013, 7: 33

Abstract

The use of information-based measures to assess changes in conscious state is an increasingly popular topic. Though recent results have seemed to justify the merits of such methods, little has been done to investigate the applicability of such measures to children. For our work, we used the approximate entropy (ApEn), a measure previously shown to correlate with changes in conscious state when applied to the electroencephalogram (EEG), and sought to confirm whether previously reported trends in adult ApEn values across wake and sleep were present in children. Besides validating the prior findings that ApEn decreases from wake to sleep (including wake, rapid eye movement (REM) sleep, and non-REM sleep) in adults, we found that previously reported ApEn decreases across vigilance states in adults were also present in children (ApEn trends for both age groups: wake >REM sleep >non-REM sleep). When comparing ApEn values between age groups, adults had significantly larger ApEn values during wakefulness than children. After the application of an 8 Hz high-pass filter to the EEG signal, ApEn values were recalculated; the number of electrodes with significant vigilance state effects dropped from all 109 electrodes with the original 1 Hz filter to 1 electrode with the 8 Hz filter. The number of electrodes with significant age effects dropped from ten to four. Our results support the notion that ApEn can reliably distinguish between vigilance states, with low-frequency sleep-related oscillations implicated as the driver of changes between vigilance states. We suggest that these observed differences between adult and child ApEn values during wake may reflect differences in connectivity between age groups, a factor which may be important in the use of EEG to measure consciousness.

Introduction

Recent theoretical work has proposed a link between the ability of the brain to integrate information and its corresponding conscious state (Balduzzi and Tononi, 2008; Tononi and Sporns, 2003; Tononi, 2012, 2008, 2004). Meanwhile, related experimental work has shown a link between changes in informational processing and conscious state (Casali et al., 2013; Ferrarelli et al., 2010; Massimini et al., 2007, 2005). These studies have provided compelling evidence of a causal relationship between the complexity of neural responses to external stimulation, as measured with the electroencephalogram (EEG), and the conscious state of the subject. Nevertheless, the benefits (particularly in the clinical setting) of a metric for conscious state independent of external stimulation are enough to encourage further work toward such a measure.

In this search for an EEG-specific measure of consciousness, many information-based measures have been applied. For this study, we chose the approximate entropy (ApEn), a measure of regularity in the time domain. Originally designed for use on physiological data (Pincus, 1991), ApEn quantifies the predictability of a signal by comparing the number of matching sequences of a given length with the number of matching sequences one increment (time bin) longer. It has been suggested as an EEG measure of conscious state, and ties into informational theories of consciousness. Theoretical analysis has shown that isolated systems should show decreases in ApEn values (Pincus, 1994). This concurs with findings that non-rapid eye movement (NREM) sleep, associated with decreases in consciousness (Stickgold et al., 2001), tends to feature long-distance connectivity decreases and increases in local clustering (Ferri et al., 2008, 2007; Massimini et al., 2007, 2005; Spoormaker et al., 2010; Uehara et al., 2013). Rapid eye movement (REM) sleep, a state similar to wakefulness in its content of conscious experience, tends to show functional connectivity patterns more similar to those of wake (Massimini et al., 2010).

Prior applications of ApEn as a measure of conscious state have successfully shown correlations with anesthetic depth (Bruhn et al., 2003, 2000a, 2000b; Hayashi et al., 2012; Li et al., 2008; Rezek and Roberts, 1998; Zhang et al., 2001), though these findings were contradicted by Jordan et al. (2006), who failed to report certain key parameters. Burioka et al. (2005) applied ApEn to data from adults across wake and sleep, finding a consistent decrease in ApEn from wake to sleep, with the lowest values occurring during deep sleep. Gu et al. (2003) also applied ApEn to data across multiple stages of sleep, and during epileptic seizure onset, reporting decreases during sleep and during seizure onset, but did not use any statistical testing. Attempts to tie ApEn

changes to behavioral changes during wakefulness have found conflicting results: ApEn analysis of subjects driving while sleep deprived found no significant changes in ApEn preceding driving errors (Papadelis et al., 2007a), though Flores Vega et al. (2013) recently showed that ApEn could be used to differentiate between some of the various mental tasks tested. Papadelis et al. (2007) found no significant changes in ApEn as a function of hypoxia, but ApEn derived metrics did show significant changes. In summary, though its resolution within the wake state is unclear, when analyzing subjects between wakefulness and other conscious states, ApEn values consistently decreased with loss of

consciousness. Comparisons of ApEn with other information-based measures typically showed it to be of comparable accuracy and reliability (Abásolo et al., 2008; Anier et al., 2012; Bruhn et al., 2003; Li et al., 2008; Rezek and Roberts, 1998; Zhang et al., 2001).

Past work has documented changes in EEG power across development, during both sleep (Buchmann et al., 2010; Feinberg and Campbell, 2010; Feinberg, 1982; Kurth et al., 2010b) and wake (Whitford et al., 2007). To our knowledge, no group has yet applied ApEn to the EEG data of children. Therefore, to further assess the merits of ApEn as a measure of conscious state, we applied ApEn to EEG data recorded across sleep and wake, from both adults and children. Besides replicating the finding that ApEn can mark changes in vigilance state due to sleep in adults, we sought to verify that similar ApEn trends were present across wake and sleep in children, while also assessing any impact of age on ApEn values across both wake and sleep.

Materials and Methods

Subjects

For this study, subjects were pooled into two age groups of six subjects each, hereafter referred to as adults (age range: 19.4–25.1 years; mean age ± SD: 23.2 ± 2.06 years; 0 females), and children (age range: 10.6–12.6 years; mean age ± SD: 11.4 ± 0.691 years; 2 females). Subjects wore wrist actigraphs and kept sleep diaries to ensure sleep schedule compliance. Napping, alcohol consumption, and taking medication were all forbidden for the 24h preceding the recording. Informed written consent was obtained from all subjects or their legal guardians. All procedures were performed with approval of the local ethics committee, and in accordance with the Declaration of Helsinki.

Data acquisition

All data (EEG, electrooculogram, and electromyogram) were gathered previously by our group at the University Children's Hospital Zurich during one evening, night, and morning. All sleep data used were originally published in earlier studies from our group (Kurth et al., 2012, 2010b). Of the data recorded previously, subjects within the selected age range and with minimallyartifacted data were used, particularly during wakefulness and REM sleep. Wake data have not yet been used for publication, and were recorded during an auditory oddball task that was performed shortly before and after full night sleep recording. Subjects were awoken at a time allowing for normal school or work attendance. A 128-electrode high-density EEG array (Electrical Geodesics, Eugene, OR, USA) was used for recording, with a sampling frequency of 500Hz. Electrodes were referenced to the vertex during recording, which was used for filtering, downsampling, and artifact rejection. Impedences were set below 50k. Data were divided into 20s epochs, the sleep stages of which were categorized using standard criteria (Iber et al., 2007). For the scoring of sleep stages, the recordings were referenced to the mastoid electrodes.

For analysis with the ApEn algorithm, data were then bandpass filtered at frequencies of 1 and 35Hz, respectively, and downsampled to 250Hz before being corrected for artifacts. Artifact correction for sleep data involved visual inspection of the power between 0.75–4Hz, and 20–30Hz, rejecting individual channels for a given epoch if the power exceeded a mean band power value. Artifact correction for wake data was based on independent component analysis, as presented by Jung et al. (2000). Finally, data were referenced to the average activity of all non-rejected channels above the ears for analysis. To investigate better the role of low-frequency EEG activity on ApEn, we later refiltered our original data with an 8Hz high-pass filter, and recalculated the ApEn.

ApEn analysis used all 109 electrodes above the ears not rejected during artifact correction. Data preprocessing and all analyses were done using Matlab (The MathWorks, Natick, MA, USA), statistical testing used Matlab, as well as R (R Foundation for Statistical Computing, Vienna, Austria). Data series of 4000 points, corresponding to 16s of EEG signal, were used for analysis. Because wake epochs were scored in epochs of 4s duration, analysis used aggregate 16s epochs comprised of four consecutive artifact-free epochs, taken from the evening recording session preceding sleep. Sleep data was drawn from the first 16s of unartifacted 20s epochs. Sleep epochs used were preceded and followed by at least 1min (three epochs) of sleep all of the same stage, to minimize the influence of stage transitions. For

one adult subject, only two epochs (40s) preceded and followed the epoch for the N3 sleep stage used for all analyses.

Approximate Entropy (ApEn)

The development of ApEn was driven by the need for a distribution-free measure of signal regularity. Unlike the Shannon entropy, the calculation of ApEn is not predicated on the underlying distribution of the data; it is instead based on sequence recurrence. This allows ApEn to be applied to signals of shorter length, and makes model estimation wholly unnecessary, removing the risk for misestimation based on poor model selection.

ApEn can be understood as the logarithmic ratio of component-wise matching sequences from a signal of length N. The other relevant parameters are r, a factor based on the standard deviation of the signal being analyzed, and used for comparison. The final parameter is m, the length of sequences compared. It is measured as an integer count of discrete time bins. The ApEn is computed as follows:

1. The first sequence of length m, is compared with all other sequences of the same length in a point-wise manner. Those sequences for which all points are within r of their corresponding point in the original sequence are counted as a match (including the base sequence with itself). This count is used in step 3.
2. The same comparison is made for sequences of length $m + 1$, starting with the first sequence of $m + 1$ points. This count is used in step 3.
3. The count from step 2 is divided by step 1, and the naturallogarithm of this ratio is taken.
4. This process is then repeated for all possible sequences (thefinal m points of the signal cannot be used, as there would be no $m + 1$ sequence for comparison).
5. All logarithm results are then summed, divided by $N - m$ (the total number of possible base sequences), and multiplied by −1.

The minimum value for ApEn is 0, suggesting a fully predictable sequence. ApEn values are heavily dependent on parameter choice, and values calculated with different parameter choices cannot be compared. Because the filter factor, r, typically has its values pegged to the standard deviation of the sequence, the origin of ApEn's robustness to noise and scale invariance can be seen. Our parameters were set per the suggestion of Pincus and Goldberger (1994), as well as other groups applying ApEn to EEG data (Bruhn et al., 2000a; Hayashi et al., 2012; Li et al., 2008), specifically Burioka et al. (2005), to m and r values of 2 and $0.2 \cdot SD$, respectively. Our N value, the length of the data series used, was 4000 points.

To confirm the proper functioning of the ApEn algorithm, we computed ApEn values for six regular sine curve sequences of 4000 points, with a simulated sampling rate of 250Hz. The sine frequencies used were 1, 2, 4, 8, 16, and 32Hz, frequencies all within the range used in our EEG ApEn analysis. Each sine curve sequence was then randomly shuffled twenty times. ApEn values were calculated for all six sine curve sequences, and all 120 random sequences (twenty random ApEn values per sine curve).

For an excellent appendix detailing the steps in ApEn calculation (including a simple by-hand walkthrough of the steps involved in ApEn calculation, as well as a sample implementation in Visual Basic), please see Bruhn et al. (2000).

Statistical Analysis

As mentioned above, statistical analyses were performed using Matlab and R. Values were imported into R and log-transformed, to better approximate a normal distribution. A linear mixed model for the subject age groups (independent factor) and vigilance states (repeated-measures factor) was then generated and tested using a repeated-measures ANOVA.

All multiple comparisons corrections were performed using the Holm–Bonferroni method. Because EEG electrodes are not independent, the Holm–Bonferroni correction is overly conservative. For this reason, in order to provide the most informative results, p-values and significance results from comparisons using all electrodes are reported both with and without correction. To better investigate differences between age groups, unpaired independent-samples t-tests were performed between each age group within each vigilance state.

Results

As described above, we analyzed a set of simulated data to validate our ApEn algorithm. ApEn values for the simulated data ranged between 0.07 and 0.29 for the sine curves. Mean ApEn values for the shuffled sequences were all 1.94, with standard deviations of less than 0.01. These results were in line with expectations.

Figure1 shows the topographical distribution of mean ApEn per electrode in adults and children. ApEn value trends across vigilance states were similar for both age groups, and were as follows: wake ApEn > REM sleep ApEn > N2 sleep ApEn > N3 sleep ApEn, though REM sleep and N2 sleep were often overlapping, especially in children. Figure2 displays the ANOVA results for all factors. All 109 tested electrodes had significant vigilance state effects after post-hoc correction. Ninety-three electrodes, widely distributed across the scalp, showed a significant age effect before correction. Ten electrodes had a significant age effect after correction. These ten electrodes were largely clustered over the left parietal and the area between the occipital and temporal lobes, with one isolated over the right temporal lobe.

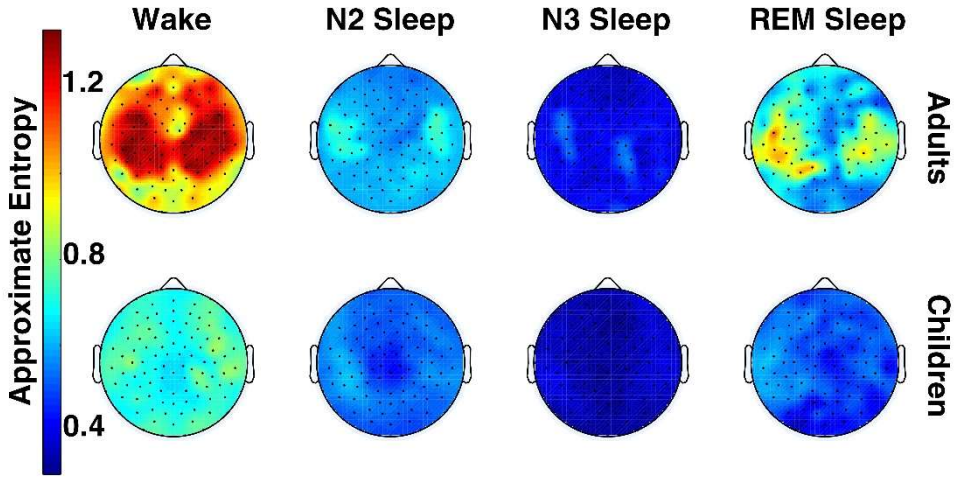

Figure 1

Topography of across-subject mean ApEn values across vigilance state in adults and children (for both groups, N=6).

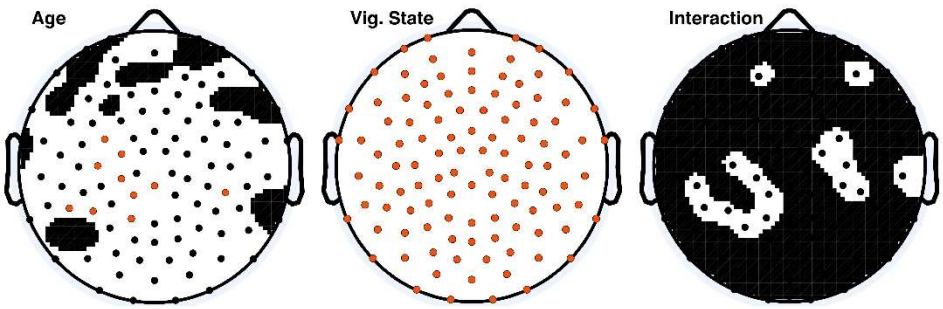

Figure 2

ANOVA p-value distributions; electrodes with factor effect p-values less than 0.05 are those displayed in black on the white background. Electrodes with significant p-values following Holm–Bonferroni correction are depicted in orange. The nasion electrode (not shown) was significant for the vigilance state effect post-hoc, and was not significant at any level for the other effects.

To better discern the causes of the observed age effects, withinvigilance state pairwise t-tests were calculated across all electrodes. These results are shown in Figure3, where 66 electrodes had significant age effects during wakefulness before correction, of which 28 electrodes were still significant following correction. N2 sleep and REM sleep had large clusters of significant electrodes before correction; none were significant after correction.

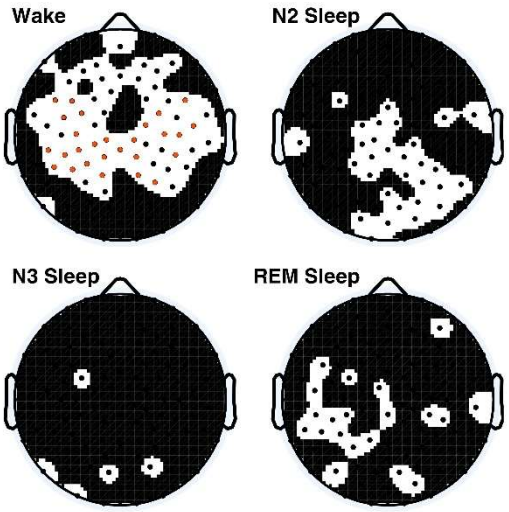

Figure 3

Within vigilance state t-test p-values across all electrodes, electrodes for which $p<0.05$ are displayed in black on the white background, values significant following Holm-Bonferroni correction are depicted in orange. The nasion electrode (not shown) was significantly different during wakefulness after post-hoc correction.

To fully explore the possibility that sleep regulatory differences between age groups may influence our results (Carskadon and Acebo, 2002; Carskadon et al., 1980), and to verify that ApEn wake values are not influenced by potential changes in overall synaptic weighting during sleep [as proposed by Tononi and Cirelli (2003)], we compared ApEn from both the evening and morning recording sessions, averaged across all 109 electrodes. A Two-Way ANOVA for age and recording session found a significant age

effect ($p < 0.001$), as expected from earlier testing, but found no significant effect for the recording session, nor for the interaction of the two ($p > 0.1$ for both effects). For all other ApEn analysis, evening wakefulness was used to represent wakefulness.

To assess the origin of the observed ApEn differences between children and adults, a high-pass filter of 8Hz was applied to the data, and ApEn values were again calculated. Two-Way ANOVA results from the high-pass-filtered data of all electrodes are depicted in Figure4. Forty-two electrodes had significant age effects before correction, of which four electrodes were significant following correction. Vigilance state effects were almost entirely abolished; seven electrodes were significant before correction; one electrode was significant after correction.

Figure 4

ANOVA p-value distributions from 8 Hz high pass filtered data; electrodes with factor effect p-values less than 0.05 are those displayed in black on the white background. Electrodes with significant p-values following Holm-Bonferroni correction are depicted in orange. The nasion electrode (not shown) was not significant at any level for any effect.

To check for changes in the regional distribution of ApEn, electrode values were normalized to the within-subject-withinvigilance-state mean across all electrodes. One electrode (located near the posterior end of the right frontal area) showed a significant vigilance state effect after correction. No other electrodes were significant for any effect (age, vigilance state, or the interaction of the two), even before post-hoc correction.

Finally, to investigate individual differences in ApEn values, we averaged ApEn across all electrodes, and plotted values for each stage as Figure5. The minimum values from wakefulness were invariably higher than the maximum observed ApEn value from sleep (including both NREM and REM sleep) within the same age group. Comparison of all subjects showed some adult sleep values (especially during REM sleep) greater than some or all wake ApEn values for children.

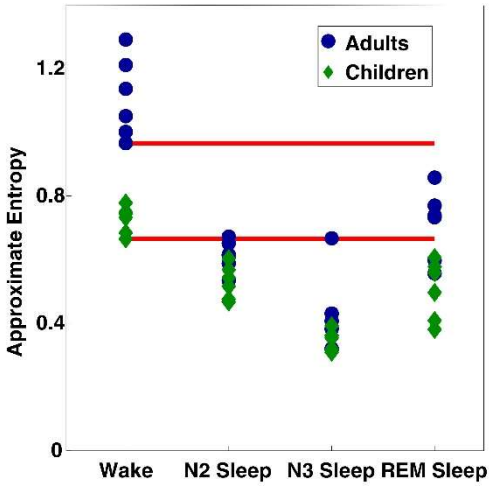

Figure 5

Scatter plot of ApEn values per subject per vigilance state, averaged across all 109 electrodes. Red bars indicate the minimum values observed for each group during wakefulness.

Discussion

Our analysis showed significant ApEn effects due both to vigilance state and age, with age differences being predominantly driven by differences during wakefulness. As a measure of vigilance state, ApEn showed strongly significant results across wake and sleep, with ApEn values in adults following the same trend as those previously reported (Burioka et al., 2005). ApEn results from children followed similar trends between vigilance states, with the only significant age differences occuring during wakefulness. As demonstrated in Figure5, within age group minimum ApEn values for wakefulness were higher than maximum ApEn sleep values for the same age group, supporting the notion that ApEn can reliably detect changes in vigilance state. The almost complete abolition of significant vigilance state effects observed following application of the 8Hz high pass filter to our data provide evidence that slow wave activity, the key EEG oscillation of deep (NREM) sleep (Buzsaki, 2006; Steriade et al., 2001), is also the key driver behind the increased regularity observed during sleep.

Pincus (1994) observed that isolated systems have lower ApEn values. If the brain is indeed a more segregated one during NREM sleep, as suggested by experimental work (Massimini et al., 2007, 2005), then one would expect to see decreases in ApEn during NREM sleep, as we did. These findings concur with the proposal presented in Tononi and Massimini (2008), which drew a link between slow wave activity during deep sleep and an interruption in information processing, leading to loss of consciousness. That ApEn differences due to vigilance state mostly disappeared after the removal of the lower frequency bands connects ApEn changes to the presence of sleep oscillations, specifically slow waves. Our results therefore suggest the possibility of a causal relationship between EEG signal changes, as measured via ApEn, and the hyperpolarization phase associated with the slow oscillation (Steriade et al., 2001). This hyperpolarization has been implicated in the induction of loss of consciousness (Massimini et al., 2005).

The almost complete lack of significant vigilance state differences following normalization to the mean value across all electrodes indicates that changes in ApEn values across wake and sleep are not the result of changing topographical distribution. These results were therefore unlike previously observed age-dependent topographical changes in sleep slow wave activity (Kurth et al., 2010b), and rather suggest that changes in signal regularity are of a more global nature.

Besides the widely distributed nature of changes due to sleep stage, changes between wake adults and children were also found to be global: Pairwise t-tests found a broad distribution of electrodes with significant increases in wake ApEn values across development. These results concur with those of Gasser et al. (1988), who found absolute EEG band power decreases in the delta and theta bands (both of which were below 7.5Hz), and the overall spectrum, across adolescence when measuring during eyes-closed wake. Our findings also agree with the EEG results of Whitford et al. (2007), who found global power decreases during wakefulness across age, especially in the lower frequency range (0.5–7.5Hz).

While EEG power changes between adults and children have also been observed during sleep [as reviewed in Feinberg (1982); Feinberg and Campbell (2010), also Buchmann et al. (2010); Kurth et al. (2010)], we only observed age differences in ApEn values during wakefulness. This discrepancy may potentially be explained by the large increase in EEG power during sleep. EEG power differences caused by sleep-related oscillations may be of a large enough scale relative to those due to developmental changes that ApEn age differences are obscured. Figure5, the scatter plot of individual mean ApEn values shows a tendency for ApEn values to be lower in children during sleep (the largest ApEn values for any given stage are invariably from adults; the lowest from children), even though statistical testing reveals no age differences.

Our results from wakefulness may also be in line with this claim; if ApEn age differences during wakefulness reflect anatomical connectivity changes, then the lack of significant differences at occipital and temporal electrodes is in line with what would be expected based on prior developmental research work. The review of Feinberg (1982) drew parallels between their work measuring changes in sleep EEG activity across development, and anatomical work, which showed regional variation in synaptic densities across development [Huttenlocher (1979); Huttenlocher et al. (1982), expanded in Huttenlocher and Dabholkar (1997)]. These works independently demonstrated that primary sensory cortices were first to reach adult-level values, both when measured via EEG power during sleep, and histological synaptic density counts. Coupled MRI and EEG work from our group found correlations between slow-wave activity decreases during sleep and gray matter volume decreases (Buchmann et al., 2010). Similar work during wakefulness from other groups showed correlations between gray matter volume decreases and low-frequency EEG decreases from late childhood through adulthood (subjects ranged between 10 and 30 years of age, Whitford et al., (2007)), particularly in the parietal and frontal regions, where our significant differences were focused. Developmental changes in the topographical distribution of low-frequency sleep oscillations followed similar trends; regions converging to adult-level synaptic densities earlier were also the first to converge to adult-level EEG activity (Kurth et al., 2010b). Without the use of other tools, such as single-unit recording or transcranial magnetic stimulation, it is difficult to separate EEG slow wave activity from the changes in functional connectivity observed on the neuronal level during slow wave sleep. Nevertheless, the decrease in ApEn observed between wakefulness in children and in adults matches with the increased local anatomical connectivity observed in children. That changes in both vigilance state and sleep result in decreased ApEn values supports the notion that changes in ApEn values may reflect connectivity changes, both anatomical and functional.

Though this claim must be further tested, if true, it would mean that ApEn changes reflect both functional (between wake and sleep) and anatomical (across development) connectivity changes in the brain. As we have shown, ApEn can reliably distinguish between wake and sleep within subject age groups. However, having demonstrated that age has an uneven influence on ApEn values across changes in vigilance state, we highlight the need for future research to fully explore the influence of age on proposed information-based EEG measures of consciousness.

Acknowledgement

The authors would like to thank Salomé Kurth, Maya Ringli, and Anja Geiger for data collection; Caroline Lustenberger, Daniel Heersink, and Mattia Molinaro for statistical advice; and David Balduzzi for helpful input and discussion

4.4 Spike wave location and density disturb sleep slow waves in patients with CSWS (continuous spike waves during sleep)

Bigna K. Bölsterli Heinzle[1,2,3], Sara Fattinger[1,2,3], Salomé Kurth[4,5], Monique K. LeBourgeois[5], Maya Ringli[4], Thomas Bast[6,7], Hanne Critelli[1,2], Bernhard Schmitt[1,2,3]*, Reto Huber[1,3,4*]

[1] Interdisciplinary Centre of Sleep Medicine, University Children's Hospital Zurich, Switzerland

[2] Division of Clinical Neurophysiology, University Children's Hospital Zurich, Switzerland

[3] Children's Research Centre, University Children's Hospital Zurich, Switzerland

[4] Child Development Centre, University Children's Hospital Zurich, Switzerland

[5] Sleep and Development Laboratory, Department of Integrative Physiology, University of Colorado Boulder, CO, USA

[6] Paediatric Neurology, University Children's Hospital, Heidelberg, Germany

[7] Epilepsy Centre Kork, Kehl, Germany

* these authors contributed equally

Financial support was provided by the Children's Research Center Zurich (to Bigna Bölsterli Heinzle), the Swiss League against Epilepsy (SLgE, to Sara Fattinger), the Anna Müller Grocholski-Foundation and the Swiss National Science Foundation (PP00A3-114923 to Reto Huber). We thank Jakob Heinzle for helpful comments.

Own contribution: analysed the data, wrote and edited the manuscript

Published in
Epilepsia 2014, 4: 584-591

Summary

Objective: In CSWS (continuous spike waves during sleep) activation of spike waves during slow wave sleep has been causally linked to neuropsychological deficits, but the pathophysiological mechanisms are still unknown. In healthy subjects, the overnight decrease of the slope of slow waves in NREM (non rapid eye movement) sleep has been linked to brain recovery to regain optimal cognitive performance. Here, we investigated whether the electrophysiological hallmark of CSWS, the spike waves during sleep, is related to an alteration in the overnight decrease of the slope, and if this alteration is linked to (1) location and (2) density of spike waves.

Methods: In a retrospective study, the slope of slow waves (0.5-2Hz) in the first and last hour of sleep (19 EEG-electrodes) of 14 CSWS-patients (3.1-13.5years) was calculated. (1) The spike wave "focus" was determined as the location of highest spike amplitude and (2) the density of spike waves as spike wave index (SWI).

Results: (1) There was no overnight change of the slope of slow waves in the "focus". Instead, in "non-focal" regions, the slope decreased significantly. This difference in the overnight course resulted in a steeper slope in the "focus" compared to "non-focal" electrodes during the last hour of sleep. (2) Spike wave density was correlated with the impairment of the overnight slope decrease: The higher the SWI, the more hampered the slope decrease.

Significance: Location and density of spike waves are related to an alteration of the physiological overnight decrease of the slow wave slope. This overnight decrease of the slope was shown to be closely related to the recovery function of sleep. Such recovery is necessary for optimal cognitive performance during wakefulness. Thus, we propose the impairment of this process by spike waves as a potential mechanism leading to neuropsychological deficits in CSWS.

Key words: Electrical status epilepticus during sleep (ESES), epilepsy, cognition

Introduction

CSWS (continuous spike waves during sleep) is characterised by variable forms of neuropsychological impairments associated with a striking activation of spike waves during slow wave sleep (Caraballo et al., 2013; ILAE, 1989; Tassinari et al., 2005). Behavioural (Perez et al., 2008) and metabolic studies (Maquet et al., 1995) point to a relationship between the location and the density of spike waves during sleep and cognitive or behavioural deficits. It is generally accepted that interictal spike waves play a causative role in the development of these neuropsychological problems with CSWS (Holmes and Lenck-Santini, 2006; Tassinari and Rubboli, 2006). However, the pathophysiological mechanisms are largely unknown.

Slow waves are the most obvious characteristics of NREM (non rapid eye movement) sleep in the surface EEG. When the majority of cortical neurons in a certain region synchronously alternates between excessive neuronal firing and neuronal silence, slow waves become detectable in the EEG as large amplitude waves below 4.5 Hz (Vyazovskiy et al., 2009). The ability of a neuronal network to synchronise its activity is linked to the strength of cortico-cortical connections, i.e. synaptic strength, in this assembly of neurons (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007b). It has been shown that the slope of EEG slow waves best reflects the efficiency to synchronise cortical neuronal networks (Riedner et al., 2007; Vyazovskiy et al., 2009). Therefore, the slope of slow waves serves as an indirect, but reliable EEG marker of synaptic strength. As the slope of slow waves decreases during sleep (Riedner et al., 2007), it was suggested to reflect a decrease in net synaptic strength.

CSWS is exemplary for an epileptic syndrome with activation of spike waves during slow wave sleep and impairments of higher cognitive functions. Notably, the syndrome occurs during childhood, the age at which slow waves are most pronounced (Feinberg, 1982; Kurth et al., 2010a) and synaptic density is highest (Huttenlocher, 1979b). Additionally, it has been shown experimentally, that the physiological slow waves turn into epileptic spike waves with increasing synchrony of slow neuronal oscillations (Steriade and Amzica, 1994). The decrease of slow wave activity during sleep has been tied to sleep homeostasis (Borbély and Achermann, 2011) and cortical recovery which is necessary to regain optimal performance during the day (reviewed in Tononi and Cirelli 2006; Vyazovskiy and Harris 2013). Taken together CSWS can be seen as a model disease for “slow wave pathology“. Supporting this view, we have demonstrated an altered physiological overnight course of the slope of slow waves during sleep in central derivations in children with CSWS (Bölsterli et al., 2011). We concluded that this alteration reflects an impairment of cortical recovery and speculated that this could be a potential mechanism for the neuropsychological deficits in CSWS. However, a link between these slope changes and the continuous spike waves during slow wave sleep, the electrophysiological characteristic of CSWS, was not established.

Thus, in the current study, we hypothesised that (1) location and (2) density of spike waves are related to the (1) topography and (2) extent of the alteration of the overnight decrease in the slope of slow waves in CSWS. To investigate these hypotheses, we retrospectively analysed 14 EEGs of patients with CSWS. We compared the slope of slow waves at the location of highest spike wave amplitude and the

remaining cortical regions in the first and the last hour of NREM sleep and related the number of spike waves, expressed in a spike wave index (SWI), to the overnight slope changes.

Methods

Subjects:

EEG recordings of children diagnosed with CSWS were identified retrospectively from databases in Zurich (1994-5/2012; 37 patients with 129 EEGs) and Heidelberg (1999-2009; 29 patients with 58 EEGs). The selection of EEGs was based on the following criteria:

- one overnight EEG with at least 30 minutes wakefulness before sleep onset and after awakening
- generalised, bilateral, focal or multifocal spike waves in over 50% of the first 30 minutes of NREM sleep
- activation of spike waves during NREM sleep (≥50% increase between last 30 minutes awake and first 30 minutes asleep)
- no treatment with corticosteroids/ACTH at the time of the EEG
- no convulsive/non-convulsive status epilepticus during wakefulness
- no tonic seizures
- a maximum of 90 minutes of continuous wakefulness between sleep onset and awakening
- over 90% accuracy of automatic spike detection (see Supplementary Methods S1)

Fourteen EEGs from 14 patients with CSWS met all criteria: 13 from Zurich and one from Heidelberg. The sample included seven females and males (age 3.1 to 13.5 years, mean 7.4 years). Four EEGs (#11, #12, #13, #14) have been included in Bölsterli et al. (2011).

All patients had at least one magnetic resonance image (MRI) obtained for clinical indications. Because neuropsychological data were not assessed in a systematic manner, a statistical correlation to the EEG features was not feasible. Each patient was age-matched to one healthy control subject (age 2.4 to 13.9 years, mean 7.1 years; Kurth et al. 2012).

The study took place at the University Children's Hospital in Zurich (Switzerland) and was approved by the institutional review board.

EEG recordings and preprocessing

Patients

EEG recordings were acquired by Neurofile/Coherence (IT- med GmbH, Usingen, Germany). The original data were exported and further processed with MATLAB (The MathWorks Inc., Natick, MA).
The recordings included at least 21 EEG derivations according to the international 10-20-system (FP1, FP2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T3, T4, T5, T6, FZ, CZ, PZ, M1, M2). One EEG was recorded with a standard modified 10-10-montage, where the same electrodes were analysed except for M1 and M2, which were replaced by low temporal derivations T9 and T10. Electromyogram and electrooculogram (EOG or FP1-FP2) were used as additional channels for sleep stage scoring.

The original signal was sampled at 1000 Hz (0.016-300 Hz) and stored after digital anti-aliasing with either 128 Hz or 256 Hz. The EEG from Heidelberg was sampled with 256 Hz (0.16-97 Hz). The data

originally stored with a sampling rate of 256 Hz were filtered with an anti-aliasing filter (low-pass at 57 Hz, two-way least-squares finite impulse response filter) and downsampled to 128 Hz. Visual and semi-automatic artefact removal was performed (Huber et al., 2000). Channels with too many artefacts were excluded (only in one patient an entire channel had to be removed). Then, the signal was re-referenced to the average of the remaining derivations out of FP1, FP2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T3, T4, T5, T6, FZ, CZ and PZ. Because of profound EEG alterations in CSWS, standard sleep stage scoring criteria were adapted as in Bölsterli et al. (2011), based on suggestions of Landolt et al. (2006). Only three stages were assigned: “Wakefulness”, “NREM sleep” and “REM-like”. If visual inspection allowed assignment of stage 1 it was done according to standard criteria (Rechtschaffen and Kales, 1968).

Control subjects

All night sleep EEGs were recorded using high density EEG (Electrical Geodesics Sensor Net, 128 channels; Kurth et al. 2012). The signal was referenced to CZ and sampled at 500 Hz (0.01-200 Hz). The same channels as in the patients (without reference electrode CZ) were selected for analysis. Further preprocessing was done as in patients.

EEG Analyses

Assessment of epileptic focus and spike wave index (SWI)

To study topographical aspects of spike waves and their relationship to the slope of slow waves we defined the epileptic focus as the derivation with the highest spike amplitude seen in the source derivation (Hjorth, 1975) at various times during the first 30 minutes NREM sleep. If the maximal amplitude shifted, different electrodes were ascribed as “focus”. Therefore, the epileptic “focus” was composed of one or more electrodes that could be neighbours or, in the case of multifocal activity, were placed more distantly from each other. The electrodes not constituting to the “focus” were ascribed as “non-focal”. In the control EEGs the respective electrodes were also named “focal” or ”non-focal”.

The density of spike waves was quantified in a SWI according to Aeby et al. (2005). SWI was calculated as the number of seconds affected by spike waves divided by the total number of seconds multiplied by 100. The SWI was determined for the 30 minutes wakefulness before falling asleep, the first 30 minutes NREM sleep and the 30 minutes of NREM sleep before awakening.

Automatic slow wave detection

We used the same signal processing procedures as described in Bölsterli et al. (2011) based on an adapted version of a slow wave detection algorithm published by Riedner et al. (2007) Individual waves (negative peaks of 0.5-2 Hz waves) were detected for each average referenced EEG derivation. All wave detection procedures were identical for patients and controls. In patients, however, the automatic detection of slow waves resulted in some spike wave complexes being erroneously detected as slow waves. In order to analyse the slope of “true” (not spike contaminated) slow waves, all waves being part of a spike wave complex were excluded. To this end, the semi-automatic spike detection algorithm previously published (Bölsterli et al., 2011) was further automatised. The final algorithm provided an objective and evaluator-independent, reliable spike detection. To assure a spike detection minimum of

90%, the algorithm was adjusted to rather “overdetect” spikes instead of an exact spike counting. A detailed description of the algorithm of spike detection and exclusion of slow waves being part of a spike wave complex is provided in the Supplementary Methods (S1).

Calculation of the slope of slow waves

For the selected waves the slope was determined as the amplitude of the negative peak divided by the time between the negative peak and the following zero-crossing. The amplitude and the slope of waves are highly correlated, i.e. the larger amplitude slow waves show steeper slopes (Kurth et al., 2010a). Therefore, we controlled the slope for amplitude differences. The slope at the amplitude of 75 μV for the first and the last hour of NREM sleep was calculated as in Bölsterli et al. (2011). This approach led to two single slope values for each electrode. All individuals exhibited slow waves with an amplitude of 75 μV in both time windows. The main results would not change if other amplitudes (e.g. 50 μV) were used. In this manuscript we will use the term ‘slope’ for the slope of the slow waves at the amplitude of 75 μV.

Statistical comparisons

All statistical analyses were performed with non-parametric tests. To test whether the “focus” of the spike waves is related to changes in the slope of slow waves, we calculated the mean slope across all electrodes constituting the epileptic “focus” and the mean slope of the “non-focal” electrodes. This approach resulted in a single value (14 values in total) for the “focus” as well as for the “non-focal” electrodes (for the first and last hour of NREM sleep for each EEG recording). For within subject comparisons a Wilcoxon-signed-rank-test was used. The overnight change was quantified in percentage by calculating the relative change from the first to the last hour. For the correlation between SWI and overnight changes in slope Spearman’s rank correlations were calculated. Overnight slope changes of “focal” and “non-focal” electrodes in patients and the respective electrodes in control subjects were compared using Mann-Whitney-U-tests. Correction for differences in duration of REM sleep between patients and controls was performed with univariate analysis of variance (covariate: REM duration (%)). Statistical significance was determined as $p<0.05$.

Results

Conventional EEG characteristics:

The mean SWI decreased from 84.0% (standard deviation (SD) 14.3%, 55.5-99.3%) during the first 30 minutes of NREM sleep to 72.0% (SD 22.0%, 25.9-99.4%) during the last 30 minutes (p=0.001). SWI was 31.3% (SD 15.9%, 0-55.0%) during the 30 minutes before sleep. Epileptic "foci" were assigned to parietal (six EEGs; P3 and P4 in one of those), central (five EEGs), temporal (two EEGs), and frontal or frontopolar (five EEGs; F3 and F4 in one of those) electrodes. Note that the total number of epileptic "foci" is higher than the number of patients because five patients had either shifting maximal amplitudes or multifocal spike waves (see Supplementary Table S1).

Sleep parameters were calculated between the beginning of the first and the end of the last hour of NREM sleep. Mean total sleep duration (TSD) and percentage of NREM sleep and wakefulness were not significantly different between patients (TSD: 505 minutes, SD 80 minutes; NREM: 72.1%, SD 10.7%; awake: 10.0%, SD 6.8%) and controls (TSD: 504 minutes, SD 68 minutes; NREM: 69.3%, SD 8.5%; awake: 5.4%, SD 5.0%). Controls spent significantly more time in REM sleep (controls: 21.3%, SD 6.2%, patients: 13.2%, SD 6.0%; p=0.002).

Clinical characteristics:

In seven out of 14 patients (#1, #4, #7, #8, #9, #12, #13) brain imaging was normal. Patients #5 and #14 showed slight generalised atrophy, patient #6 a mild focal subcortical and paraventricular gliosis and patient #10 a discrete bilateral paratrigonal signal alteration. Three patients (#2, #3, #11) had posthaemorrhagic lesions (two of them had hydrocephalus treated with ventriculoperitoneal shunts). Statistical comparisons to electrophysiological parameters were not feasible because the study group was too small and diverse.

Although neuropsychological data were not assessed in a systematic manner and thus not allowing any statistical comparison, the patients could be described roughly. In 11 out of 14 patients developmental regression was specified either in the patients' history or by neuropsychological testing (#1, #4-9, #11-14). Patient #3 with posthaemorrhagic hydrocephalus scored repeatedly around IQ 70 in variable testing batteries. However, three years after the analysed EEG, neuropsychological testings yielded improvement (>1 SD) in executive functions, working memory and processing speed. And these changes coincided with an improvement of the sleep EEG. Patient's #2 early cognitive development seamed normal beside a hemiparesis. After the diagnosis of CSWS, feasible neuropsychological testing was not possible because of behavioural problems. In patient #10 variable testings revealed IQ between 40 and 50. Primary developmental delay was evident and aetiology was unknown but likely to be syndromic or genetic (unspecific MRI abnormalities and slight dysmorphic features). The girl's teachers reported developmental stagnation shortly after the detection of CSWS.

Topography of spike waves and local changes in the slope of slow waves

In a first step, the relationship between the location of the most prominent spike waves (i.e. the "focus") and the overnight changes in the slope of slow waves was addressed (see Figure 1, 2 and Supplementary Table S1). While we found no overnight change in the slope over the "focus", the slope

declined significantly in the “non-focal” electrodes (p=0.035). We observed no difference in the slope between “focal” and “non-focal” electrodes at the beginning of the night. But at the end of the night the slope was significantly steeper over the “focus” compared to the “non-focal” electrodes (difference 7.1%, p=0.008). Consequently, the relative change of the slope from the first to the last hour tended to be different in the “focal” (+1.25%) compared to the “non-focal” electrodes (-3.02%, p=0.074). Moreover, the slope decrease in the patients’ “non-focal” electrodes was still significantly smaller than in the control group (p<0.001). As expected, our control group showed an overall decrease (e.g. mean of all electrodes) of the slope of slow waves across the night (p=0.001). In contrast to the patients, the control subjects exhibited no difference of the overnight slope decrease between the electrodes equivalent to the “focal” and “non-focal” electrodes of the patients (mean “focal”: -27.9%, mean ”non-focal”: -27.5%). The individual variability of this decrease was similar in “focal” and “non-focal” electrodes as well as in patients and controls (Figure 2).

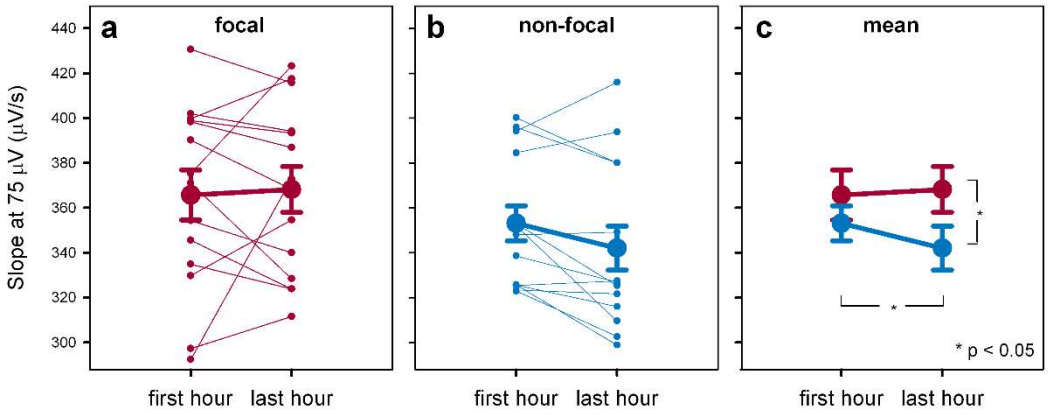

Figure 1

(a-c) Overnight changes of the slope of slow waves. Ascending slopes at the amplitude of 75 µV (see Methods) are shown for the 14 averaged “focal” (left) and “non-focal” derivations (middle) (light circles and lines). Bold, darker lines indicate the group mean. Error bars illustrate s.e.m. In the right panel the mean slope (±s.e.m.) of “focal” (red solid line) and “non-focal” electrodes (blue solid line) are superimposed. Stars indicate significant differences (p < 0.05, Wilcoxon-signed-rank-tests).

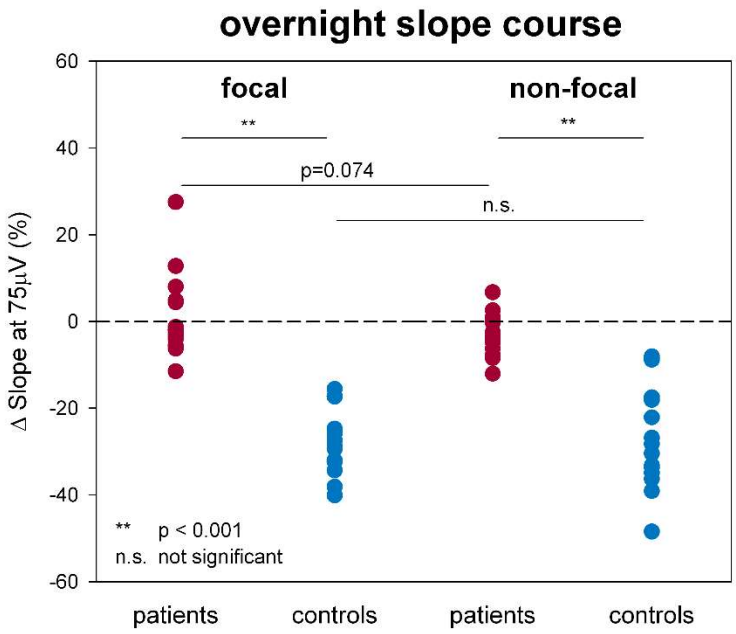

Figure 2

Overnight slope decrease in patients (red dots) and controls (blue dots). “Focal” and “non-focal” electrodes of the patients’ EEGs and the equivalent electrodes of the control EEGs are plotted. Note that all control EEGs exhibit an overnight slope decrease (dots below dashed line).

Density of spike waves and overnight changes in the slope of slow waves

In a final step we investigated the relationship between the density of spike waves (SWI) and the overnight change in the slow wave slopes. We found a positive correlation between the SWI during the first 30 minutes NREM sleep and the overnight slope change (absolute and relative change). In other words, the denser the spike waves at the beginning of sleep, the more hampered was the physiological overnight decrease of the slope that is observed in healthy subjects and is necessary for normal brain function. The strongest correlation was found for the overnight slope change in the “focus” (Rho=0.68, p=0.008, Figure 3). Hence, the negative effect of the spike density on the slope decrease was most pronounced in the location of most pronounced spike waves (“focus”). Moreover, the overall mean slope change (across all electrodes; Rho= 0.66, p=0.01) and the mean slope change in “non-focal” electrodes (Rho=0.57, p=0.032, Figure 3) also correlated with the SWI in the first 30 minutes NREM sleep. Finally, the SWI before awakening correlated significantly with the overnight slope change in the “focal” electrodes only (Rho=0.57, p=0.034).

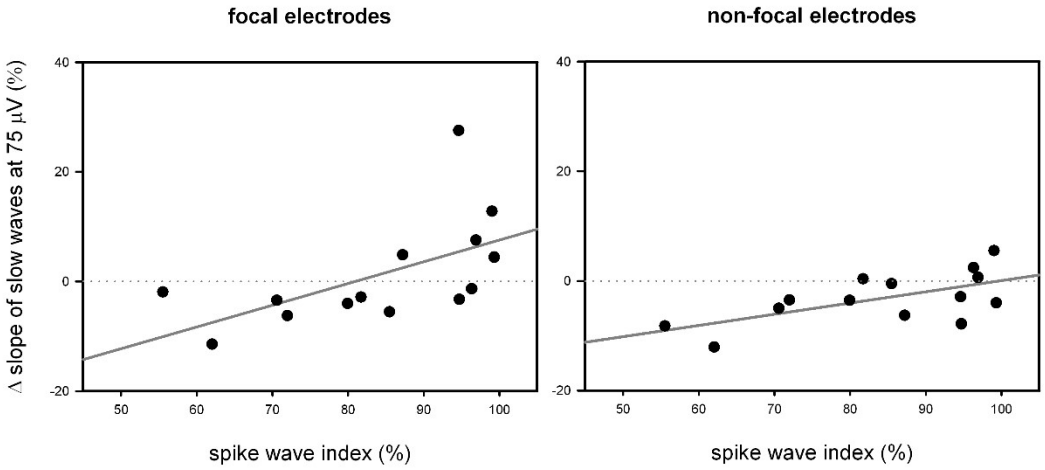

Figure 3

Correlations between the relative overnight changes in slope and SWI in the first 30 min NREM sleep in “focal” electrodes (left) and ”non-focal” electrodes (right). Negative Δ slope stands for an overnight slope decrease, positive Δ slope for an increase. Note that many subjects with very high (>85%) SWIs showed a slope increase, i.e. the symbols above the dotted grey line. The solid grey line indicates a linear regression curve. Spearman’s Rho = 0.68, $p<0.01$ (“focal”), Rho = 0.57, $p<0.05$ (“non-focal”).

Discussion

In this study we provide first evidence that the previously demonstrated disturbance of the decrease of the slope of slow waves in CSWS (Bölsterli et al., 2011) is directly related to the spike waves..

- First, we found the strongest impairment of the slope decrease to be in the location presenting the highest amplitude spike waves.
- Second, the denser the spike waves were the more hampered was the physiologically observed slope decrease.

There are various reports of a close relationship between the spike wave focus in CSWS and specific neuropsychological impairments (Landau and Kleffner, PhD, 1998; Perez et al., 2008; Veggiotti et al., 1999). For example, individuals with CSWS showed higher glucose metabolism (measured by FDG-PET) in the epileptic focus during both sleep and wakefulness, with the region of the focus in agreement with the modality of the neuropsychological deficits (Maquet et al., 1995).

In healthy subjects, slow waves can be induced by a task specific activation of brain regions during wakefulness and have been correlated to task performance improvements after sleep (Huber et al. 2004; reviewed in Lustenberger and Huber 2012). Thus, a link between a local regulation of slow waves and specific performance has been established. Based on this, Tassinari & Rubboli (2006) hypothesised that prolonged focal epileptic activity in CSWS interferes with local slow wave activity, and that this interference might affect cognitive functioning. Our data lend support to this hypothesis. We found a relationship between the location of the most prominent spike waves and the topographical distribution of the slope changes. More specifically, electrodes in the epileptic “focus” showed no overnight change in the slope of slow waves. Instead, in “non-focal” electrodes, a slope decrease was observed, which however, was smaller compared to controls. Hence, the slope change across the night was altered not only in the main epileptic regions but also in more distant cortical areas.

In the surface EEG, slow waves can be characterised by various parameters, i.e. slow wave activity (SWA), amplitude and slope. It has been shown that the slope of slow waves best reflects neuronal synchrony of subjacent neuronal networks (Vyazovskiy et al., 2009). The efficiency to synchronise neuronal networks closely depends on synaptic strength (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007b). Thus, the slope of slow waves serves as an indirect EEG measure of synaptic strength. To exclude a potential confound due to an influence of the spike on the following slow wave we performed the slope analysis only for slow waves that are not part of a spike wave complex. An additional advantage of using the slope is it can be calculated for individual slow waves. SWA in contrast is calculated for frequency bands and is based on a continuous EEG signal. As a consequence, the specific analysis of selected slow waves not being part of spike wave complexes would not have been possible.

A recent hypothesis about the function of slow waves, the synaptic homeostasis hypothesis (Tononi and Cirelli, 2006), predicts that synapses are strengthened during wakefulness and weakened during sleep.

Strengthening of synapses, the neuronal process of learning, needs energy and space. To save energy and space, the hypothesis predicts, that during sleep the synapses are downscaled proportionally to their strength. This leads to a preservation of the relative strength of neuronal connections achieved during wakefulness. Hence, synaptic downscaling is important for synaptic plasticity and cortical recovery. The synaptic homeostasis hypothesis yields some intriguing interpretation of our experimental findings. We conclude that a long-lasting locally pronounced disturbance of the overnight slope decrease might lead to specific cognitive impairments by interfering with local recovery and local plasticity. Poor recovery would mean poor overnight decrease in energy expenditure, in line with the finding of a higher glucose metabolism level in the epileptic focus in patients with CSWS[5]. The reduced slope decline in regions beyond the epileptic "focus" might lead to additional but less severe neuropsychological deficits, not directly related to the "focus".

The density of epileptiform dischargesduring sleep seems to play a role in the occurrence of developmental problems in CSWS (reviewed in Scheltens-de Boer 2009). Urbain et al. (2011) found impaired sleep dependent memory consolidation in four patients with activation of spike waves during sleep. A single patient showed a normalisation of the EEG after treatment and interestingly, also regained sleep dependent memory improvement. The decrease of the slope of physiological slow waves has been linked to sleep dependent memory improvement (Tononi and Cirelli, 2006). By manipulating slow waves, a causal relationship between slow waves and sleep dependent memory improvement has been established in healthy subjects (Landsness et al., 2009; Marshall et al., 2006; Ngo et al., 2013). For example, the enhancement of slow waves by auditory closed-loop stimulation applied in-phase with slow oscillations led to an improvement of sleep dependent consolidation of declarative memory. The "boosted" slow waves showed an increase in slope. And a more pronounced overnight slope decrease was found (Ngo et al., 2013).. In contrary, a suppression of slow waves by acoustic stimulation abolished the physiological sleep dependent performance improvement in a visuomotor learning task (Landsness et al., 2009). Thus, it can be speculated that the spike wave associated impairment of sleep dependent memory improvement might be driven by a spike wave dependent alteration of the physiological slope decrease. In agreement with such an interpretation, we found a positive correlation between the SWI and the overnight slope change. Interestingly, in subjects with a very high SWI, roughly above the originally proposed cut-off SWI of >85% for CSWS35, even an overnight increase of the slope was observed. As the slope of slow waves reflects synaptic strength, this finding implies an increase of net synaptic strength. This interpretation of our data supports the concept of spike dependent synaptic potentiation (Buzsáki, 1989; Holmes and Lenck-Santini, 2006).

To alleviate the problem of a small sample size, we performed a retrospective analysis of patients from two centers and set the cut-off SWI for inclusion at 50%. This percentage has been proposed as diagnostic criteria and was related to developmental regression (reviewed in Scheltens-de Boer 2009). We paid attention to count the SWI systematically and used a well-described method (Aeby et al., 2005). To ascertain that the pathology was sleep related, a 50% increase of SWI from wake to NREM sleep was required (Sánchez Fernández et al., 2012). Only high quality EEGs, which could be reliably analysed in the first and the last hour of NREM sleep were included, leading to some drop outs (12/26).

Although the sample size was rather small, our results reached significance and, importantly, are in line with previous findings on a different (four out of 14 EEGs overlapping) EEG sample (Bölsterli et al., 2011).

Because of the retrospective study-design, antiepileptic treatment was not standardised. However, the fact that multiple drug combinations were administered renders a systematic bias unlikely. Moreover, EEGs recorded during treatment with corticosteroids were excluded, because direct influences of these substances on slow waves (Holsboer et al., 2008) and cognition (Wilhelm et al., 2011) have been described. Only one patient had received corticosteroids prior (more than one year) to the analysed EEG. To “blind” the analyses, the determination of the epileptic “focus” and the SWI were performed by different co-authors than the calculation of the slope of slow waves.

The retrospective study-design did not permit to establish a relation between overnight slope changes and neuropsychological features. Hence, the interpretation of our results that the impaired physiological overnight course of the slope is the cause for cognitive deficits in CSWS remains speculative. Prospective studies with standardised neuropsychological testing at the time of EEG recordings are needed to study this link. In addition, to improve our understanding of the relation between the impairment of sleep dependent learning and changes in the overnight course of the slope of slow waves, learning tasks need to be included. In any case, the difficulty to test patients with severe cognitive impairments remains.

The age of onset of CSWS is around 4 to 8 years (Caraballo et al., 2013; Nickels and Wirrell, 2008), when synaptic density is increasing (Huttenlocher, 1979b), and the syndrome dissolves around puberty when synapses are pruned. However, the neuropsychological impairments often outlast the electrophysiological normalisation. These enduring impairments seem to be most pronounced when CSWS starts at a young age and is long-lasting (Smith and Hoeppner, 2003). Animal experiments provide evidence for an age dependent impact of spike waves on cortical plasticity. Young rabbits with Penicillin-provoked subclinical focal spiking in visual cortex developed anatomical changes in visual networks, while these changes could not be triggered in adult rabbits (Baumbach, 1981). Synaptic plasticity is use dependent and physiological use occurs during wakefulness. “Use”, however, depends on behaviour only during wakefulness, while during sleep, synaptic downscaling might be a driving force for synaptic competition. One could speculate that in CSWS the winner of the competition are the synapses “used” in epileptic networks and consequently pathologic networks would be tightened.

Conclusion: This study establishes a relationship between the location and density of spike waves and an alteration of the physiological decrease of the slope of slow waves during sleep, which has been linked to recovery and cognition. We conclude that the alteration of the slope decrease might be the basis for the neuropsychological deficits, and the local alteration for the modality of the deficits. In contrast to the concept of transient cognitive impairment by interictal epileptic activity that is measured during wakefulness, our findings suggest a spike dependent pathophysiological mechanism for the development of cognitive impairments in CSWS that is linked to sleep.

Supplementary Methods

Patient #	Epileptic „foci“	Slope first hour	Mean slope first hour	Slope last hour	Mean slope last hour
1	PZ	354,3		340,0	
2	P3	398,4		386,9	
3	P3	339,0	334,8	313,6	323,9
	P4	330,7		334,2	
4	PZ	345,6		323,9	
5	C4	345,4	329,8	339,9	354,6
	T5	314,1		369,3	
6	C4	292,4		372,9	
7	FP1	401,9		394,1	
8	FP1	430,7		415,9	
9	FP2	367,1	371,0	323,3	328,5
	PZ	374,9		333,6	
10	T4	297,2		311,6	
11	FP2	406,0	399,8	423,8	417,5
	P3	393,7		411,2	
12	C4	390,2		368,4	
13	CZ	375,3		423,2	
14	F3	409,6	398,9	417,5	393,4
	F4	417,9		388,3	
	C4	369,3		374,3	

Table S1

All epileptic „foci“ with their respective slope of slow waves at the amplitude of 75µV (slope) are listed. In the five patients with either shifting maximal amplitudes or multifocal spike waves, the slopes of the “focal” electrodes were averaged. This led to single values for the first and last hour of NREM-sleep for each patient (14 values in total). These values are plotted in Figure 1a and entered statistical comparison (see Methods).

Automatic spike detection

Because spikes in the EEG recordings were mostly monomorphous and their regional distribution is highly stable over the night, we computed an independent component analysis (ICA) using the EEGLAB toolbox (Delorme and Makeig, 2004) for Matlab. The ICA was performed separately on the first and last hour of average referenced, artefact-free NREM sleep EEG. Independent components that delineated the time course of spikes (out of 19 components) for the first and the last hour of NREM sleep as follows:

We first high-pass filtered (4 Hz) all components and then automatically detected the negative half-waves with a zero-crossing to zero-crossing time interval in the range between 31 and 86 ms (i.e. 4 to 11 samples), which is in the frequency range of spikes (5.82 to 16 Hz). The detected negative half-waves were sorted according to their absolute amplitudes and visualised in a histogram. In these histograms, ICA components describing spikes were characterised by a bimodal distribution of wave-amplitudes with many small amplitude waves reflecting the background noise, almost no waves at

amplitudes of intermediate magnitude and many high amplitude waves reflecting the spikes. In order to ensure good estimates of the shape of the histogram, only histogram containing at least 100 waves per bar were accepted for this analysis. We established an automatic procedure to determine the bimodality which was based on the following criteria: The highest bar or the first local maximum had to be located within the 20 percent of smallest amplitudes. At intermediate amplitude waves a “valley” (local minimum) had to occur. Finally, at high amplitude waves the second local maximum or the “spike-mountain” had to occur. The latter was defined as the sum of all the waves with higher amplitudes than the ones of the “valley”-bar and had to contain at least 150 waves. A component fulfilling these criteria for a bimodal amplitude distribution was determined as a “spike-component”. In a final step, for each of these “spike-components” we visually checked in a topoplot the regional distribution of the maximal amplitudes of the component. These locations were compared to the conventionally assigned focus. At least one “spike-component” matching to each epileptic focus had to be found. If this was not the case we increased the resolution (number of bars) in the amplitude histogram. However, 5 multifocal EEGs had to be excluded because the “spike-components” only described parts of the conventionally defined spike locations. Only ICA components fulfilling all criteria mentioned above were subjected to the second step of the spike detection procedure.

As a next step, we defined an amplitude threshold for spike detection. The threshold was based on the histogram which had a bimodal shape. We defined the amplitude threshold relative to the minimum between the first and second maximum of the histogram. From this minimum (“valley”-bar) we subtracted 5% of the distance to the first maximum, which resulted in a value between the first maximum and the minimum but was much closer to the minimum. For each component, all waves with a peak above this threshold were detected as spikes. Please note, that it was possible that one spike was described by peaks detected in different independent components and thus had more than one time sample assigned to it.

After the automatic spike detection, all negative peaks of the originally detected slow waves occurring within 0.5 s of a detected spike were excluded from further analysis. The accuracy of the spike detection was evaluated visually in each EEG by comparing the automatically and visually detected spikes in the focal electrodes on seven 10-second-pages of the first and last hour of NREM-sleep (the first and last page and one page every 10 minutes). EEGs with irregular and therefore visually not exactly countable spikes were not evaluable properly and were therefore excluded for further analysis (see inclusion criteria).

4.5 Impaired slow wave sleep downscaling in patients with infantile spasms

Sara Fattinger[1,2,3,4], Bernhard Schmitt[1,2,3], Bigna K. Bölsterli Heinzle[1,2,3], Hanne Critelli[1,2], Oskar G. Jenni[1,3,4], Reto Huber[1,3,4,5]

[1] Pediatric Sleep Disorders Center, University Children's Hospital Zurich, Switzerland;
[2] Division of Clinical Neurophysiology, University Children's Hospital Zurich, Switzerland;
[3] Children's Research Center, University Children's Hospital Zurich, Switzerland;
[4] Child Development Center, University Children's Hospital Zurich, Switzerland;
[5] Neuroscience Center Zurich (ZNZ), University of Zurich, Switzerland;

Financial support was provided by the Swiss League against Epilepsy (SLgE), the Swiss National Science Foundation grants 3100-05300.97, 3100A0-100567, 320030-130766 and PP00A-114923, the Human Frontiers Science Program Grant RG 0131/2000-BR102 and a research grant from the University Research Priority Program of the University of Zurich.

Own contribution: analysed the data, wrote and edited the manuscript

Published in
European Journal of Paediatric Neurology 2015, 19: 134-142

Abstract

Background: West syndrome is a severe epileptic encephalopathy of infancy, characterized by infantile spasms, global retardation, and severely abnormal electroencephalogram (EEG) pattern known as hypsarrhythmia, which is most prominent during slow waves sleep. The restorative function of slow wave sleep has been linked to downscaling, a neuronal process ensuring a balance of global synaptic strength, which is important for normal cortical functioning and development. A key electrophysiological marker for this downscaling is the reduction of the slope of slow waves across the night.

Methods: We retrospectively compared the slope of slow waves between 14 untreated patients with infantile spasms and healthy age and gender matched controls. Patients were examined in one all-night sleep EEG before treatment, and in two follow-up nap recordings, under and after treatment with corticosteroids.

Results: In patients with infantile spasms the overnight reduction in the slope of slow waves was significantly diminished compared to controls ($p=0.009$). Moreover, untreated patients revealed overall steeper slopes. During corticosteroid treatment the slope was reduced compared to controls ($p=0.001$). After successful treatment the slope was similar between patients and controls.

Conclusion: Our results provide evidence for reduced downscaling in patients with infantile spasms. Moreover, the marked reduction of the slope during corticosteroid treatment may reflect a loss of synaptic connections due to the effect of glucocorticoids. This altered sleep dependent regulation of synaptic strength in infantile spasms may contribute the underlying pathomechanism of the developmental regression. Furthermore the normalization of synaptic strength due to corticosteroids might provide a potential mechanistic explanation for this treatment strategy.

Keywords: Infantile spasms, corticosteroids, hypsarrhythmia, slope of sleep slow waves, NREM sleep

Introduction

West syndrome is one of the most malignant epilepsies of infancy, characterized by infantile spasms, hypsarrhythmia and frequent mental retardation (Hancock et al., 2009; ILAE, 1989). The long-term prognosis is often poor, although hormone therapy (i.e., Adrenocorticotropic hormone (ACTH) or oral corticosteroids) or vigabatrin (VGB) might effectively treat the spasms and often also improve or normalize the electroencephalogram (EEG). The onset of spasms is frequently associated with developmental regression and long-term cognitive impairments. West syndrome is classified as an epileptic encephalopathy (Berg and Millichap, 2013) indicating that the epilepsy and the abnormal EEG (hypsarrhythmia) contributes to deterioration of cerebral functioning. Although developmental outcome in infantile spasms is substantially determined by the underlying disease, evidence is accumulating that the severity of the hypsarrhythmia (Kramer et al., 1997) and the time to treatment (O'Callaghan et al., 2011) is related to the developmental outcome. However, so far no mechanistic explanation of such a link exists.

Under physiological conditions deep NREM sleep is characterized in the EEG by the predominant occurrence of high amplitude slow wave. During the last decades a growing body of research has demonstrated, that these slow waves can be used as an indirect but reliable marker of synaptic strength (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007b). Moreover, within the framework of the synaptic homeostasis hypothesis the restorative function of slow wave sleep has been directly linked to neuronal recovery and cortical maturation (Tononi and Cirelli, 2006). The hypothesis claims that the learning related rise in synaptic strength needs energy and space, eventually leading to a saturation of learning capacity. Hence, for a normal physiological development synaptic strength needs to be reduced (synaptic downscaling), which, according to the hypothesis, takes place during slow wave sleep. This global synaptic downscaling enables new cortical plasticity the next day and thereby increases learning capacity (Tononi and Cirelli, 2006). Synaptic downscaling, the renormalization of synaptic strength across the night, is reflected in the physiological decrease of the slope of slow waves across the night. Consequently, the slopes of slow waves at the beginning of the night are much steeper compared to the slopes of slow waves with the same amplitude towards the end of the night (Riedner et al., 2007).

Considering the severely disturbed slow wave sleep in patients with infantile spasms due to hypsarrhythmic pattern and the fact that the onset of spasms is often associated with mental retardation we were asking the question whether synaptic downscaling might be impaired in patients with infantile spasms. To test these hypotheses, we performed a retrospective case-controlled study in 16 infants diagnosed with West syndrome. We analyzed the characteristics of sleep slow waves (in particular the slope) in one all-night sleep EEG before treatment, and in two follow-up nap recordings (under and after treatment). Data were compared to healthy age and gender matched controls. We hypothesize that hypsarrhythmia during NREM sleep might impair the overnight reduction of the slope of slow waves in infantile spasms. Moreover, when slow waves sleep is normalized, after successful treatment with corticosteroids, we expect normalization in the slope of slow waves.

Methods

Participants

In a retrospective analysis untreated patients diagnosed with infantile spasms were selected from the data base of the Children's Hospital Zurich (examined between 2008-2012) according to the following clinical and electrophysiological criteria:

1. Onset of infantile spasms between 1-24 months of age
2. Infantile spasms recorded by video EEG
3. Hypsarrhythmic pattern as the prominent EEG feature during NREM sleep and ictal EEG registered during infantile spasms
4. One complete all-night EEG before treatment, a first follow-up nap under treatment and a second follow-up nap after treatment with corticosteroids

Since 2008 an all-night video-EEG is part of our regular diagnostic work up in newly diagnosed infantile spasms to register the serial spasms and possible additional convulsive and non-convulsive seizures. In total, 16 infants met the above criteria. Age ranged from 2.5 to 11.2 months (mean age 6.2 ± 0.6 months, 11 boys). One boy was excluded from the analysis because of poor quality of the overnight EEG and another one because EEG never showed hypsarrhythmia although serial infantile spasms were regularly registered in the EEG at awakening (for an overview of the included patients see Table 1). After the initial overnight sleep EEG, patients were treated with prednisolone (PRD) alone (n=3), or ACTH followed by PRD (n=4) or each combined with VGB (PRD&VGB, n=6; ACTH/PRD&VGB, n=1, see Fig 1 and Table 1). Eight patients (six boys, two girls) also participated in the on-going International Collaborative Infantile Spasms Study (ICISS www.bath.ac.uk/health/research/iciss). Dosage and duration of treatment corresponded to the treatment protocol of Lux et al., 2004 (2004). After ~ 2 weeks of treatment patients received the first follow-up nap sleep EEG (Nap 1, recording time 12:50 ± 00:36 pm). The nap EEG of all responders (no infantile spasms, disappearance of hypsarrhythmia during wake and sleep) were further analyzed (seven boys, two girls, mean age 6.4 ± 0.5 months). Non-Responders were excluded because the short sleep duration of the Nap EEG did not provide enough slow waves which were not compromised by hypsarrhythmia. After completion of treatment (3.9 ± 0.5 months after the all night EEG), patients underwent a second follow-up nap sleep EEG (Nap 2, recording time 13:27 ± 00:12 pm). One boy relapsed and one girl did not fall asleep during the nap recording and were excluded. Thus, the naps of six boys and one girl (9.6 ± 0.9 months) were analyzed (for a detailed overview see Tab 2 & 3).

Data of the patients were compared to healthy age and gender matched controls. All control infants were free of neurological or developmental disorders. EEG recordings of the control group were obtained from different sources:

Control group 1: For the overnight comparison previously presented data from longitudinal PSGs of 11 full term infants (six girls and five boys) were analyzed (for details see Jenni et al. 2004). To achieve good matching with the patient group we selected the overnight recordings from four boys and six girls.

From two boys we used three subsequent nights. These lead to a total of 14 overnight recordings for the comparison.

Control group 2 and 3: To match the nap EEG of the patients with infantile spasms (Tab 3) we selected nap EEG recordings from 15 infants (two girls and 13 boys) performed at the University Children's Hospital Zurich (1995-2012) for diagnostic routine procedures. These infants were referred to our epilepsy center because of paroxysmal events of unknown causes. None of the infants suffered from epilepsy or seizures and none was treated with anticonvulsants or any other drugs. These controls were divided into two groups (control group 2 and control group 3) corresponding to the patients age at the time of the nap recordings. Control group 2 included nine infants (seven boys and two girls, mean age 6.6 ± 0.4 months) recorded on average at 12:27 ± 00:43 pm. Control group 3 consisted of seven infants (six boys and one girl, mean age 9.5 ± 1.0 months) recorded on average at 13:22 ± 00:33 pm. The nap recording of one girl was used twice i.e. in the control group 2 and 3.

The study was conducted at the University Children's Hospital Zurich (Switzerland) and approved by the local ethics committee. The study was performed according to the Declaration of Helsinki.

EEG Recordings

All PSG of the patients and the control groups 2 and 3 were obtained by Neurofile/Coherence (Natus Europe GmbH, Munich, Germany). The EEG recordings included at least 21 channels according to the 10-20 system (FP1, FP2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T3, T4, T5, T6, FZ, CZ, PZ, A1, and A2, referenced to F3/F4 or to an additional electrode between FZ and CZ). In addition, EMG (or O1-O2) and EOG (or FP1-FP2) were used to score sleep stages. If EMG or EOG were not included in the montage, EMG from the neck muscles (O1-O2) and EOG from the prefrontal electrodes (FP1-FP2) were used. The original EEG signal was sampled at 256 Hz. After band-pass filtering (0.16 - 40 Hz) the EEG was down sampled to 128 Hz. Overnight EEG of the control group 1 were recorded with a portable polygraphic amplifier system (PS1; Institute of Pharmacology and Toxicology, University of Zurich, Switzerland). The electrodes were placed along the antero-posterior axis over both hemispheres according to the 10-20 system (bipolar derivations: F3C3, F4C4, C3P3, C4P4, P3O1, P4O2; the signal was recorded to the reference derivation C3A2). The signals were sampled at 512 Hz (0.16-70 Hz), low-pass filtered (below 30 Hz) and down sampled to 128 Hz (For details see Jenni et al. 2004).

Sleep stages were visually scored for consecutive 20-s epochs. For the healthy control groups and the nap EEGs of the patients (Nap 1 & 2) sleep stages were determined according to standard criteria (NREM sleep 1-3, REM). Overnight recordings of the patients were difficult to score according to standard criteria because of the pathological electrophysiological changes. Therefore criteria were adapted (based on suggestions of Landolt et al. 2006) similar to the ones described by Bölsterli et al. (2011). Only three different sleep stages were scored: Wakefulness, NREM sleep, and REM-like sleep. The analysis of sleep slow waves of the current work was based on NREM sleep (healthy controls N2 & N3 combined, patients NREM sleep). Therefore, the missing subdivision into N2 and N3 in the patients did not influence the results.

Data analysis

The analysis was based on the EEG derivation P3A2. However, data from the other derivations revealed similar results (data not shown); therefore only data from P3A2 are presented. Thus, the EEG signal over the left parietal cortex was re-referenced to the right mastoid (P3A2). Visual and semiautomatic artefact correction was performed based on two frequency bands (0.75 to 4.5 Hz and 20 to 30 Hz[14]) for the removal of epochs containing poor data quality.

The physiological decrease of the slope of slow waves across the night is already present at the age of 2 months (Fattinger et al., 2014). Therefore, single sleep slow waves were detected, to analyze the overnight decrease of the slope of slow waves as a marker of synaptic downscaling. Spike waves and slow frequency activity in West syndrome patients are likely to reflect altered neuronal activity of cortical neurons. Therefore, only normally shaped sleep slow waves should be analyzed to achieve a reliable comparison between West syndrome patients and healthy controls.

Since we were interested in overnight changes of synaptic strength, the beginning and the end of the all-night sleep EEG of all patients were carefully inspected by a certified EEG specialist (BS). The night sleep period was defined from the first to the last scored NREM sleep epoch. The night sleep period was considered terminated when the last NREM sleep cycle was followed by more than one consecutive hour of wakefulness (minimal sleep period in the patient group 407.7 min, in the control group 391.3 min). Only artefact free NREM epochs consisting of EEG activity without spike waves or hypsarrhythmia and lasting for at least 2 seconds (for mean duration of the hypsarrhythmia-free intervals of each patient see Table 1) were marked until ~15 min of normal NREM sleep EEG activity at the beginning and the end of the overnight EEG was collected. For the further analysis of the slope of sleep slow waves only the marked epochs were considered. Inspection of the raw EEG revealed that 15 min of normal sleep EEG were sufficient to obtain enough slow waves to perform our analysis. On average 52.1 ± 3.6 min of NREM sleep needed to be inspected to obtain 15 min of normal NREM sleep EEG. Within these 15 minutes of normal NREM sleep EEG, 611.3 ± 51.3 sleep slow waves were detected (for detection algorithm see below). To assess overnight changes in the slope of sleep slow waves of healthy controls single slow waves of the first and last hour of NREM sleep were detected (on average 2280.8± 67.6 sleep slow waves were detected). For reasons of simplification, the term first and last hour of NREM sleep is used for both groups to describe the beginning and the end of sleep. The nap EEGs of all patients were also inspected, spike wave activity was marked and excluded from the analysis.

Next, single slow waves were automatically detected applying a similar procedure as described by Riedner et al. (2007): After band-pass filtering (Chebyshev Type two Filter: pass-band 0.5 and 4.0 Hz, stop-band: below 0.16 and above 10 Hz) sleep slow waves during artifact free NREM sleep epochs (i.e. stage ≥ NREM2) were detected as negative deflections of the EEG signal between two consecutive zero-crossings. For all negative half-waves of any amplitude but within a frequency of 0.5 and 2 Hz the samples of all zero-crossings and local minima of the signal (amplitude) were identified. The ascending slope of slow waves was then defined as the amplitude divided by the time difference between the

sample of the local minimum and the subsequent zero-crossing (for a schematic illustration see Fig 1 in Kurth et al. 2010).

The slope and the amplitude of sleep slow waves are closely related (Riedner et al., 2007). The larger the amplitude of slow waves the steeper the slope. Therefore, for an unbiased assessment of synaptic strength, general amplitude differences need to be controlled. To take this into account the slope of sleep slow waves with different amplitudes were analyzed separately. The mean slope of slow wave of all detected waves within 10 µV amplitude bins was calculated for each subject. Only amplitude bins containing at least 20 waves were considered.

Statistics

All comparisons between the groups were performed for the derivation P3A2. For the comparison of the slope of slow waves across different amplitude bins between patients and healthy controls a linear mixed model ANOVA was calculated with subjects as random intercept (to account for repeated measurements of the same person), group/time and amplitude bins as fixed effects. The model was covariant with duration (to control for different time intervals between the first and last detected wave) and any sleep variable revealing group differences. If covariates were not significant within the model, they were excluded to report main effects. Paired or unpaired student-t-tests (one sided) were used to compare intra- and inter-individual differences. The significance level was set at 5%. All data are presented as mean ± standard errors [SE]. All analyses were performed using the software package MATLAB (MathWorks) or SPSS 16.0 (IBM).

Results

Sleep architecture

Sleep architecture of the overnight recordings was comparable between the healthy controls (control group 1) and patients with infantile spasms (Tab. 2). Both groups showed adequate sleep quality, with only ~ 10% wakefulness for the considered night sleep period (for details see methods). The only difference in sleep parameters was found in the amount of REM sleep: healthy controls spent more time in REM sleep compared to patients. During the nap recordings (Tab. 3), no significant differences were found between patients and controls.

#	Age [Mo]	Clinical findings at presentation	Magnetic resonance imaging	Hypsarrhythmia-free interval [s]	Treatment	Response to medication	EEG Nap1 under treatment	EEG Nap2 after treatment	Outcome
1	6.21	IS only	Normal	10.4±0.6	PRD & VGB	Yes	No Hyps No SW	No Hyps No SW	Normal
2	8.22	IS and focal seizures, minor hemiparesis right	Pontomesencephale dysplasia bilateral insular pachygyria	4.3±0.3	ACTH/PRD & VGB	Yes	No Hyps No SW	No Hyps Few SW F3	DR, hyperactive
3	7.23	IS, DR, disturbed sleep-wake rhythm	normal	5.6±0.2	PRD	Yes	No Hyps No SW	No Hyps No SW	DR
4	9.83	IS only	Dysplasia (right frontal)	5.5±0.6	PRD & VGB/others	No			IS, multiple seizures, DR
5	4.41	IS, muscle hypotonia, irritable	Normal	3.2±0.1	ACTH/PRD	Yes	No Hyps No SW	No Hyps No SW	Normal
6	5.00	IS, sniveling, occasionally "mentally absent"	Normal	2.8±0.1	PRD & VGB	Yes	No Hyps No SW	No Hyps No SW	Normal
7	3.16	IS and focal seizures	Normal	2.6±0.1	PRD & VGB	No			DR, pharmacoresistant epilepsy
8	4.01	IS only	Normal	3.8±0.1	PRD	Yes	No Hyps No SW	No Hyps SW P3	Normal
9	2.47	IS and focal seizures	Dysplasia (left temporal and occipital)	3.1±0.0	PRD & VGB	No			DR, focal seizures
10	5.26	IS, occasionally "mentally absent"	Normal	3.4±0.1	ACTH & PRD	Yes	No Hyps No SW	No Hyps No SW	DR
11	5.26	IS, only	Normal	8.8±2.6	PRD	Yes	No Hyps No SW	no Hyps no SW	Normal
12	6.41	IS, DR, muscle hypotonia, apathetic	Frontal atrophy	5.0±0.2	PRD	No			DR, muscle hypotonia
13	7.46	IS, only	Normal	3.4±0.1	PRD & VGB	Yes/Relapse	No Hyps Few SW T3		Relapse IS, DR
14	8.61	IS, only	Altered ventricle configuration	5.6±0.3	ACTH & PRD	No			DR, focal seizures

Table 1 Overview of the patients

Clinical findings at presentation, MRI findings, mean duration of the hypsarrhythmia-free interval, Treatment, response to medication, EEG at Nap 1 (under treatment), EEG at Nap 2 (after treatment), and outcome of all patients (IS = infantile spasms, DR = developmental regression, Hyps = hypsarrhythmia, SW = spike/spike waves, VGB = vigabatrin, PRD = prednisolone, ACTH = tetracosactide depot).

	Age [d]	Sex	TST [min]	NREM [min]	REM [min]	Wake [%]	WASO [min]
Control group 1	177.9±17.7	8*m/6f	518.4±25.1	300.8±19.3	187.4±11.1	9.5±1.3	37.5±7.2
Patients overnight	181.5±17.4	9m/5f	486.8±15.3	317.2±12.6	131.5±3.7	10.6±1.2	52.2±7.1
p-Value	0.44		0.15	0.24	<0.001	0.29	0.08

Table 2 All night sleep

Age, gender and sleep architecture of the overnight recordings for the control group 1 and the patients. Mean values ± SE are shown (TST = total sleep time (between the first and the last hour of NREM sleep); Wake = percentage of wakefulness for the defined night sleep period; WASO = wake after sleep onset, * from 2 boys 3 different night recordings are used).

	Age [d]	Sex	RT [min]	TST [min]	NREM [min]	REM [min]
Control group 2	200.8±12.4	7m/2f*	11.4±42.7	25.5±2.3	18.3±1.9	0±0
Patients Nap 1	195.6±14.9	7m/2f	-11.4±36.5	26.5±2.3	23.2±2.2	0.7±0.7
p-Value	0.4		0.28	0.38	0.06	0.17
Control group 3	390.0±30.8	6m/1f*	2.3±33.5	22.1±1.8	18.5±1.8	0±0
Patients Nap 2	291.4±28.4	6m/1f	-2.3±11.5	23.8±1.8	20.7±2.4	0±0
p-Value	0.49		0.4	0.26	0.23	1

Table 3: Nap 1 & 2

Age, gender and sleep architecture of the nap recordings for the control group 2 & 3 and the patients (Nap 1 & 2). Mean values ± SE are presented (RT=recording time (start time of the recording expressed as difference to the mean start time across the control group 2 and Nap 1 (12:38 pm) and across the control group 3 and Nap 2 (13:24 pm); TST=total sleep time, *the nap recording of one girl was used for control group 2 & 3).

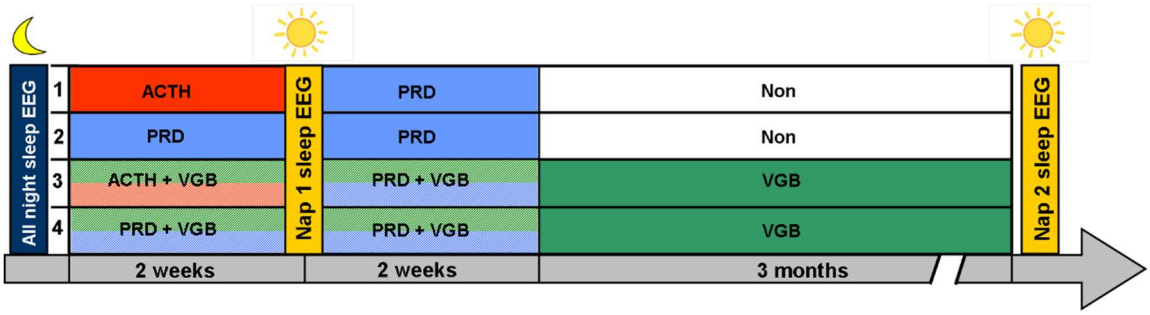

Figure 1

Overview of the EEG registrations with respect to the treatment plan. After the diagnosis of infantile spasms (according to the overnight sleep EEG) patient were treated with one of four different therapy approaches. All patients received a first follow-up nap (Nap 1) after ~2 weeks of treatment. After ~4 months (discontinuation of all medications) the second follow-up nap (Nap 2) took place (ACTH = tetracosactide depot, PRD = prednisolon, VGB = vigabatrin).

Overnight changes of sleep slow waves

To investigate overnight changes in sleep slow waves, single slow waves were detected during the first and last hour of NREM sleep (i.e., NREM stage 2 and 3, for details see Methods). The results of the following paragraph include comparisons between the overnight recordings of the patients and control group 1.

The slope and the amplitude of slow waves are closely related, the larger the amplitude the steeper the slope (Riedner et al., 2007). Meaning, for an unbiased assessment of the slope of slow waves the amplitude needs to be controlled. Therefore the slopes of slow waves with the same amplitude were calculated. In the healthy controls we found a consistent overnight decrease of the slope of slow waves from the first to the last hour of NREM sleep, independent of the amplitude (ANOVA F_{Time}=96.2, p<0.001; Fig 2 left panel). We also found an overnight decrease in the patient group (ANOVA F_{Time}=6.9, p=0.009;

Fig 2 right panel). However, this overnight decrease was significantly smaller in patients as compared to control subjects (ANOVA F_{Group}=7.9, p=0.009). The slope decreased by 10.0±1.4% on average in controls and by 4.5±2.1% in patients (Fig 2, insert).

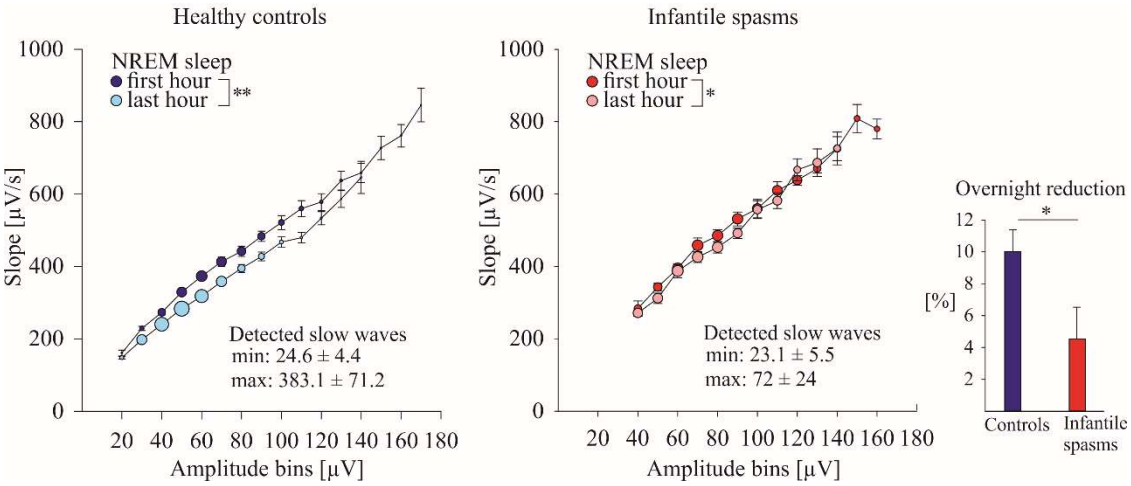

Figure 2

Slope of sleep slow waves for consecutive 10 µV amplitude bins (numbers represent the upper limit) of the first and last hour of NREM sleep for healthy controls (left) and patients with infantile spasms (right). Group mean ± SE for both time points are shown. Circle size represents the number of slow waves detected for each amplitude bin. Numbers in the lower right corner of each plot show the maximal and minimal number of slow waves detected across all subjects (mean ± SE). Note the general lower amount of detected waves in the patient group (ANOVA fixed effect Time (first hour vs. last hour) only between overlapping amplitude bins, n ≥ 7, ** p<0.001, * p=0.009). Insert: Average decrease of the slope of slow waves for healthy controls (blue) and infantile spasms patients (red; group mean ± SE, unpaired student t-test, * p=0.037).

During the first hour of NREM sleep the slope of slow waves tended to be steeper in patients compared to controls (ANOVA FGroup=3.5, p=0.07; Fig 3 left panel). Due to the impaired overnight decrease of the slow wave slope in patients, this difference reached significance when comparing the slope of slow waves of the last hour of NREM sleep (ANOVA FGroup=16.9, p<0.001). These findings were not affected by the different number of detected waves between the controls and the patients. When we calculated the slope of 282 (minimal number of detected waves in the patients) slow waves (randomly selected) of the first and last hour of NREM sleep, a similar result was found (FH ANOVA F_{Group} = 4.3, p= 0.048; LH ANOVA F_{Group} = 45.4 , p<0.001).

Effect of treatment on the slope of slow waves

After 2 weeks of treatment the first follow up nap sleep EEG (Nap 1) was recorded. Interestingly, patients revealed less steep slopes when compared to controls for slow waves with similar amplitudes, indicating a reduction of synaptic strength under treatment (ANOVA F_{Group}=14.9, p=0.001, Fig 3 middle panel). Since the duration spent in NREM sleep tended to be longer in patients compared to controls (Tab 3), we also included the amount of NREM sleep as a covariate in the model. However, the difference in the slope between patients and control could not be explained by a longer duration of NREM sleep found in patients (ANOVA F_{NREM}=0.01, p=0.98).

A second follow-up nap EEG (Nap 2) after treatment (~ 4 months after the diagnosis of West syndrome) was recorded and again compared to healthy controls (control group 3). No differences were found in the slope of sleep slow waves between controls and patients, (ANOVA F_{Group}=0.2, p=0.7, Fig 3 right panel).

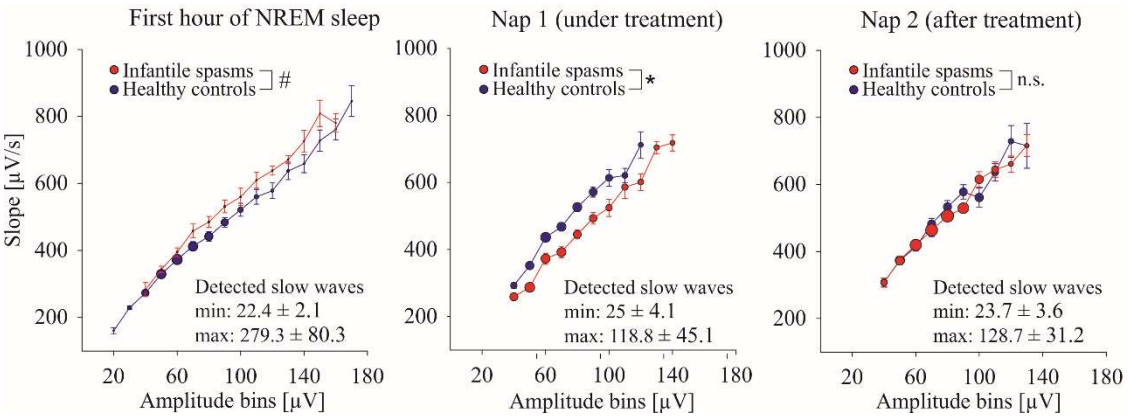

Figure 3

Slope of sleep slow waves for consecutive 10 µV amplitude bins (numbers represent the upper limit) of healthy controls (blue) and patients with infantile spasms (red) for 3 different points in time (from left to right) before treatment, under treatment, after treatment. Group mean ± SE are shown. Circle size represents the number of slow waves detected for each amplitude bin. Numbers in the lower right corner of each plot show the maximal and minimal number of slow waves detected across all subjects (mean ± SE; ANOVA fixed effect Group (healthy controls vs. infantile spasms) only between overlapping amplitude bins; n≥5, * p=0.001, # p=0.07, n.s. = not significant).

Discussion

The physiological decrease of the slope of slow waves across the night, reflecting the restorative function of sleep, was reduced in infants with infantile spasms compared to controls. Moreover, patients revealed steeper slopes, a sign of increased synaptic strength. However, during corticosteroid treatment the slope of slow waves was markedly reduced. After successful treatment, the slope of slow waves normalized, revealing similar values compared to controls.

Selection of feasible sections of physiological slow waves during NREM sleep before treatment

All included patients showed a typical, atypical or modified hypsarrhythmic pattern during a significant time period during NREM sleep of the all-night recordings before treatment. But, all patients also showed repeated periods of NREM sleep without overlying hypsarrhythmia or spike waves, periods in which physiological sleep rhythms became obvious. Because 12/14 our patients were diagnosed within 4 weeks after initiation of spasms this “fragmented” hypsarrhythmia might be due to the short diagnostic delay. In addition, 9/14 of the included patients had a normal MRI and an unknown aetiology. Those idiopathic or cryptogenic patients are known to show “fragmented” hypsarrhythmia, especially at the very beginning and as mentioned by Dalla Bernardina and Watanabe bilateral physiological background activity and well defined physiological rhythms during sleep are often recognizable between hypsarrhythmia (Dalla Bernardina B and Watanabe, 1994). Although all patients in our study have EEG registered infantile spasms and hypsarrhythmia during a significant time of NREM sleep, the density of hypsarrhythmia was different: in some patients we found NREM sleep periods without hypsarrhythmia lasting ~15 seconds, in other patients the hypsarrhythmia-free intervals were mostly between 2 and 3 seconds (for an overview of the mean duration of the hypsarrhythmia-free intervals of each subject see Table 1). Our cohort was to small to investigate the influence of hypsarrhythmia density or “spike wave index” on the overnight decrease of the slope of slow waves, as recently found in patients with CSWS (Bolsterli Heinzle et al., 2014). However, one patient of our cohort was excluded, since the EEG never showed hypsarrhythmia. Interestingly, in this patient we found an overnight decrease of the slope of slow waves which was similar to the control group (9.8%).

Sleep architecture

The sleep stage variables were comparable between patients with infantile spasms and healthy controls. In the considered night sleep period of the overnight recordings both groups showed adequate sleep quality. The good sleep quality of the patients group could be explained by the relatively normal clinical presentation at the time of recording (see Table 1). Only in one patient (# 3) an altered sleep-wake rhythm was reported. However, at the recording night of this patient only 8.4% of wakefulness was scored. As reported previously, the amount of REM sleep was reduced in patients (Hrachovy et al., 1981). No correlations, however, were found between REM sleep and overnight slope changes, both in patients and healthy controls (data not shown).

Overnight changes of sleep slow waves – before treatment

In Infantile spasms patients the decrease of the slope of slow waves across the night, a marker for the restorative function of sleep (Riedner et al., 2007), was significantly reduced, indicating impaired

synaptic downscaling. So far, several links between the slope of slow waves and synaptic strength have been reported. For example, Kurth et al. (2010) showed that the reduction in synapse density during puberty is reflected in a decrease of the slope of slow waves. Moreover, the slope follows a similar trajectory as the increasing synaptogenesis during the first year of life (Fattinger et al., 2014). According to the synaptic homeostasis hypothesis physiological slow wave sleep is pivotal for synaptic downscaling (Tononi and Cirelli, 2006). The underlying cortical slow oscillation between periods of neuronal activity and periods of neuronal silence (Steriade et al., 1993b) are functionally related to a proportional downscaling of all synapses leading to a net reduction of synaptic strength (Czarnecki et al., 2007; Lante et al., 2011). Thus, the chaotic pattern of hypsarrhythmia during slow wave sleep might impair synaptic downscaling in patients with infantile spasms.

Effect of treatment on the slope of slow waves

After the initial diagnosis all patients were treated with corticosteroids (PRD or ACTH/PRD) alone or combined with VGB (PRD&VGB or ACTH/PRD&VGB) and a first follow up nap was recorded (still under treatment). The sleep EEG of all responders was further analyzed. Interestingly, beside the elimination of hypsarrhythmia the slope of slow waves was markedly reduced. Assuming that synaptic strength is reflected in the slope of slow waves, the pronounced decrease of the slope might reflect a loss of synaptic connections due to an effect of glucocorticoids in the brain. Indeed, the potential for high dosage of glucocorticoids to induce dendritic atrophy and synapse loss in the cerebral cortex and hippocampus has been reported in animal models (Brown et al., 2005; Cook and Wellman, 2004; Liston and Gan, 2011; Tata and Anderson, 2010; Wellman, 2001). In addition, there are several case reports in the literature reporting cerebral atrophy during corticosteroid treatment. The atrophy was reversible after cessation of medication (Bentson et al., 1978; Gordon, 1980; Maekawa et al., 1980). Since no MRIs during and after treatment are available of our patient sample, a causal relationship between cortical atrophy (as a possible consequence of synaptic loss) and reduced slope values remains speculative.

After successful treatment of hypsarrhythmia and discontinuation of all medications (after ~4 months), the slope of slow waves was similar between patients and controls indicating a normalization of synaptic strength. However, whether the normalization of the slope of slow wave is indeed related to an improved overnight synaptic downscaling in patients with infantile spasms can only be speculated, since only nap sleep EEGs were available under and after treatment. Furthermore, the different level of the homeostatically regulated sleep pressure during daytime and nighttime (Achermann and Borbély, 2003) does not allow a direct intraindividual comparison of the slope of slow waves before and during/after treatment. Moreover, it would be interesting to know, whether the regulation of the slope of slow waves was still impaired in the non-responder group. Unfortunately all non-responders had to be excluded, since the short sleep duration of the nap including hypsarrhythmia did not provide enough slow waves for the analysis.

Several neurological disorders have been associated with infantile spasms, which certainly contribute to the developmental impairments in these patients. However, besides the broad heterogeneity, all

patients included in the current study, suffered from hypsarrhythmia during a significant period of NREM sleep. Moreover, the disappearance of hypsarrhythmia was associated with a normalization of the slope of sleep slow waves. The analysis of the current work was based on normal sleep slow waves (i.e., not associated with epileptiform activity). Our findings show that the slope of slow waves can be used as an electrophysiological marker to investigate signs of altered neuronal properties independent of epileptiform discharges. Thus, direct comparisons between clinical and healthy population are feasible.

Limitations and outlook

Discussing these results some additional limitations need to be considered. The low number of patients included in our study prevents any comparisons between the different treatment approaches. Thus, the influence of the treatment approaches (i.e., corticosteroids alone or combined with VGB) on synaptic homeostasis needs to be investigated in a larger sample. Furthermore, for the overnight comparison, there is an imbalance in the gender matching between control group 1 and the patient group (control group 1: 8 boys; patient group: 9 boys). Our goal was to match the patient group as close as possible regarding gender and age. Since we have shown a strong influence of age on the slope of slow waves (Fattinger et al., 2014), but not gender (control group 1: unpaired Student t-test $p=0.46$), our primary criterion for the matching was age and not gender. We do not believe that this imbalance has any influence on our results since no difference between boys and girls has been found.

Conclusion

In conclusion, our analysis provides evidence for impaired synaptic downscaling due to hypsarrhythmia in patients with infantile spasms. Successful treatment with corticosteroids seems to reduce synaptic strength supporting a normalized regulation in the balance of synaptic strength. Such a normalization of synaptic homeostasis seems to be essential, because it is thought to be important for brain development and cerebral functioning during the day. Proportional downscaling of all synapses may reduce energy and space demands while maintaining the relative synaptic weights and thereby increases the efficiency of neuronal functioning (Tononi and Cirelli, 2006). Hence, an impaired synaptic downscaling resulting in an altered synaptic balance might contribute to the developmental regression seen in these patients.

4.6 Deep sleep maintains learning efficiency of the human brain

Sara Fattinger[1,2]*, Toon T. de Beukelaar[3]*, Kathy L Ruddy[4]*, Carina Volk[1,2], Natalie C. Heyse[1,2], Joshua A. Herbst[5], Richard H. R. Hahnloser[3,5], Nicole Wenderoth[2,3,4]**, Reto Huber[1,2,6]**

[1] Child Development Center, University Children's Hospital Zurich, Switzerland.

[2] Neuroscience Center Zurich (ZNZ), University of Zurich, Switzerland.

[3] Movement Control and Neuroplasticity Research Group, Department of Kinesiology, KU Leuven, Belgium.

[4] Neural Control of Movement Lab, Department of Health Sciences and Technology, ETH Zurich, Switzerland.

[5] Institute of Neuroinformatics, University of Zurich and ETH Zurich, Switzerland.

[6] University Clinics for Child and Adolescent Psychiatry, University of Zurich, Switzerland.

* Equal contribution

** Equal contribution

Financial support was provided by grants from the Swiss National Science Foundation (project number 320030-153387 and 320030-149561) and from the Research Foundation – Flanders (grant numbers G.0401.12). TTdB. is a predoctoral fellow of the Research Foundation Flanders and was additionally supported by a JUMO grant (14/042).

Own contribution: conceived the experiment, performed the measurements, analysed the data, wrote and edited the manuscript

Resubmitted to
Nature Communications

Abstract:

It is hypothesized that deep sleep is essential for restoring the brain's capacity to learn efficiently, especially in regions heavily activated during the day. However, causal evidence in humans has been lacking due to the inability to sleep deprive one target area while keeping the natural sleep pattern intact. Here we introduce a novel approach to focally perturb deep sleep in motor cortex, and investigate the consequences on behavioral and neurophysiological markers of neuroplasticity arising from dedicated motor practice. We show that the capacity to undergo neuroplastic changes is reduced by wakefulness but restored during unperturbed sleep. This restorative process is markedly attenuated when slow waves are selectively perturbed in motor cortex, demonstrating that deep sleep is a requirement for maintaining sustainable learning efficiency.

Introduction

Many of us know from personal experience that a single night of low quality sleep can make mental tasks effortful and inefficient. Accordingly, one theory proposes that sleep is crucial for restoring the brain's metabolic (Xie et al., 2013) and neural homeostasis (Tononi and Cirelli, 2014; Vyazovskiy and Harris, 2013), thus ensuring efficient functioning during the next bout of wakefulness. Environmental inputs are constantly experienced when awake and lead to a progressive increase of synaptic strength (Huber et al., 2013; Vyazovskiy et al., 2008). Perpetual increases in synaptic strength, however, would render the brain highly insensitive to new inputs because neurons would lose their ability to fire selectively and synapses could not be further potentiated, thus saturating neural plasticity (Turrigiano, 2008). Additionally, the need for cellular maintenance (Vyazovskiy and Harris, 2013) and the removal of potentially neurotoxic waste (Xie et al., 2013) would be markedly enhanced causing an unsustainable level of energy consumption. Deep sleep in particular is thought to be essential for down-regulating synaptic strength (Tononi and Cirelli, 2014). During deep non-rapid eye movement (NREM) sleep neurons start to oscillate between a depolarized on-state when they fire and a hyperpolarized off-state when they are silent (Steriade, 2003). Because neurons are highly interconnected, synchronization of on- and off-states within larger neuronal assemblies drives 0.5-4.5Hz oscillations, termed "slow waves", which are typically detected in the surface electroencephalogram (EEG) or local field potentials during NREM sleep (Vyazovskiy et al., 2009). Slow wave activity (SWA, EEG power between 1-4.5Hz) is highest shortly after falling asleep, i.e. when the sleep need is still high, while it is markedly reduced at the end of the night after restorative processes have taken place (Borbély and Achermann, 2011). However, until now the proposal that SWA is necessary for restorative processes has been mainly supported by correlative evidence demonstrating that neural plasticity induced while awake (e.g. by practicing a specific task) leads to more SWA during sleep (C. Hanlon et al., 2011; Huber et al., 2004). This effect is highly localized with SWA being significantly higher in brain areas that were activated during the task than in non-task areas, suggesting that the brain responds to a locally increased need for sleep (Huber et al., 2004; Hung et al., 2013; Tononi and Cirelli, 2014; Vyazovskiy et al., 2008). However, in order to demonstrate that slow waves are directly responsible for restorative processes, one has to establish a *causal* relationship between these phenomena. Such an endeavor has thus far proved impossible due to the inability to manipulate SWA in humans on a local level. Here, we introduce a novel perturbation approach where real time closed-loop acoustic stimulation was timed to coincide precisely with the vulnerable down-phase of EEG slow waves (Vyazovskiy and Harris, 2013). We targeted SWA in primary motor cortex (M1), taking advantage of well-established behavioral and neurophysiological markers of motor training-induced neuroplasticity to estimate the effect of locally perturbed deep sleep in M1.

Results

We report data from 13 volunteers (21.2 ± 0.4 years of age, all right handed, 6 females) who were first familiarized with the procedures and participated then in two experimental sessions (see Fig. S1 for subject drop-out and outlier detection). Each session consisted of three learning assessments: a new motor sequence (finger tapping) was learned on the morning of Day 1 (Mor D1), another new motor sequence was learned on the evening of Day 1 (Eve D1) which was followed by one night of sleep in the laboratory during which high-density (HD) EEG was measured, and another new motor sequence was learned on the morning of Day 2 (Mor D2) (Fig.1A). During one experimental session sleep was perturbed by acoustic stimulation triggered by the down-phase of local slow waves in M1 (STIM), while in the other session subjects were allowed a normal night of sleep (NOSTIM) (Fig. 1, A and B; see Methods). Subjects were blind to this experimental manipulation and subjective sleep ratings were not significantly different between sessions (Table S4), nor were subjective ratings of different psychological parameters (e.g. focus, motivation or tiredness, Table S3) and objective measures of vigilance (Table S5).

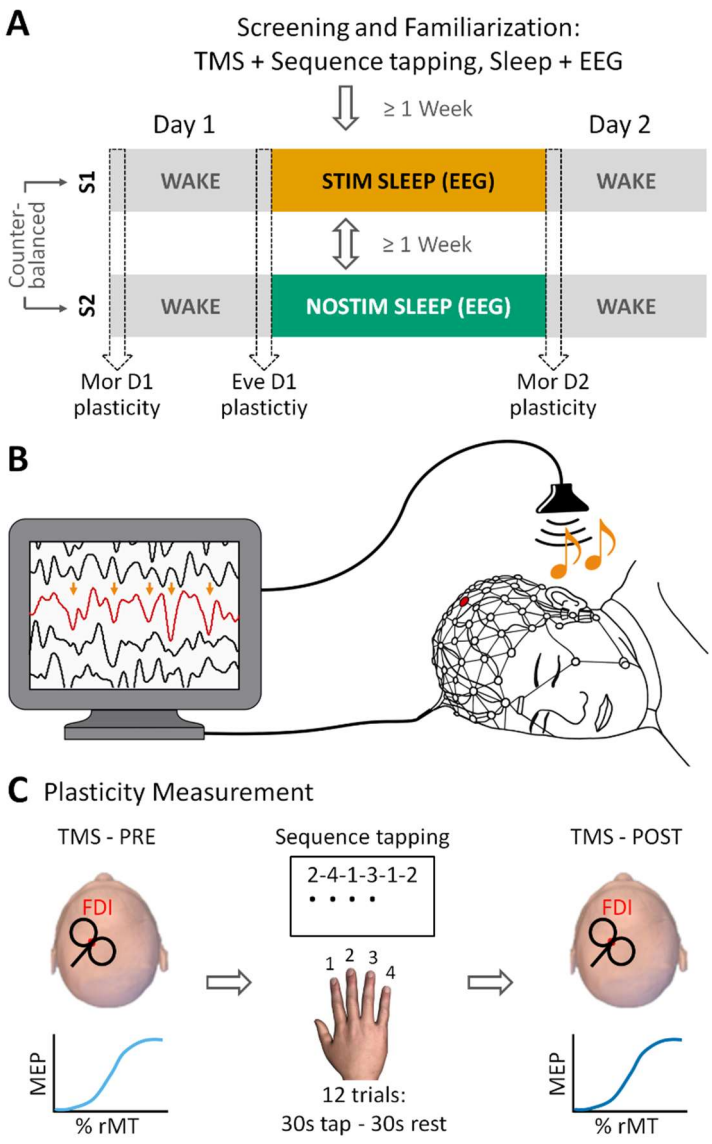

Fig. 1

Experimental protocol. (A) After a familiarization session, volunteers participated in two experimental sessions. These were separated by 1 week, with both sessions including three separate learning assessments during which motor plasticity was tested in the morning of day 1 (Mor D1), the second in the evening on the same day (Eve D1), and a third in the morning the next day (Mor D2). In one experimental session, slow waves were perturbed using acoustic stimulation (STIM, yellow) while in the other experimental session no stimulation was applied (NOSTIM, green). (B) In the STIM sessions slow waves were perturbed using acoustic stimulation precisely time-locked to the down-phase of sleep slow waves (yellow arrows) detected in the electrode located closest to the sensorimotor representation of the trained hand (red electrode and signal). (C) During each learning assessment subjects acquired a new six-element motor sequence during 12 training trials (i.e. 30s tapping followed by 30s rest) lasting 12min in total. Changes in corticomotor excitability of the right first dorsal interosseus (FDI, red dot) were measured before motor training (TMS-PRE) and after (TMS-POST) via an input-output curve (IO curve).

Acoustic stimulation was switched on during stable NREM sleep and was triggered by slow waves in the 0.5-2Hz frequency range. Stimulation caused a local reduction of SWA, most prominently in the low

frequency range (low SWA, 1-2Hz, see Fig S2). We found a 12.00 ± 3.92% power reduction in low-SWA during the STIM session compared to the NOSTIM session in a cluster of 9 electrodes (Fig. 2, for a comprehensive overview of the entire frequency range (0.5-25 Hz) see Fig. S3.) Additional analyses focused on the hotspot-electrode in M1 (for details see Methods, Fig. 1B, Fig. 6A). Low-SWA measured by this electrode was reduced by 13.4 ± 4.12% (Fig. 6, A and D; $p = 0.007$, paired t-test; n = 13). During STIM sleep we applied acoustic stimulation on 53.1 ± 3.71% of all slow waves present during the night. This caused a significant acute effect (evoked manipulation) by steepening the slope of the up-phase and shortening the duration of the perturbed slow wave (Fig. 3, A and B; $p < 0.05$, paired t-test; n = 13). The stimulation also changed general slow-wave characteristics throughout sleep (Fig. 3, C and D). Thus, the level of synchronization necessary for the generation of large amplitude waves was reduced, and as a consequence, SWA was also reduced. This finding is also supported by the positive correlation between the slope reduction of the down-phase and the reduction in low-SWA (Spearman's *rho* = 0.69, $p = 0.01$; n = 13). Exploratory analyses including all channels revealed that the general effects of acoustic stimulation are locally restricted (Fig. 3 E and F).

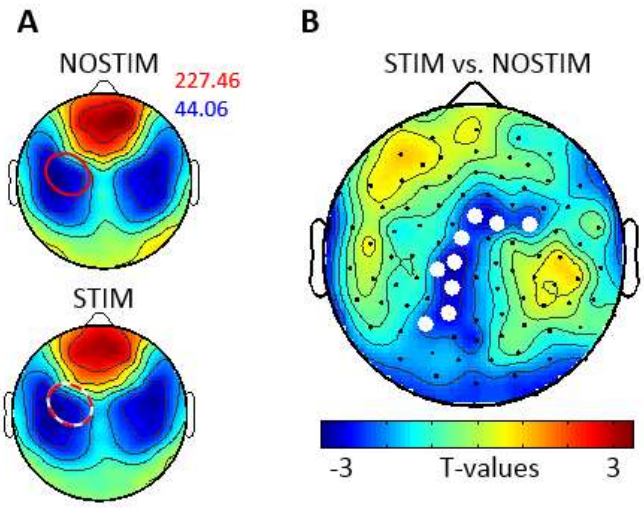

Fig. 2

Comparisons of low slow wave activity (low-SWA, 1-2Hz) between NOSTIM and STIM sessions. (A) Topographical map of low-SWA of the two experimental nights scaled to maximum (red) and minimum (blue) power values (μV/Hz2). Red circles indicate the position of the TMS hotspot, the white circle indicates the position of the selected electrode for slow wave detection. Note, in the main experiment the TMS hotspot electrodes and the selected electrodes for slow wave detection were the same. (B) Statistical comparison (t-values) of low-SWA between the STIM and NOSTIM sessions (paired t-test; n = 13). Blue colors indicate a decrease and red colors an increase in low-SWA in the STIM compared to NOSTIM session. During STIM session sleep a reduction of low-SWA of 12.00 ± 3.92% ($p = 0.009$) in a local cluster of 9 electrodes over left sensory-motor area (white dots, $p < 0.05$, after nonparametric cluster-based statistical testing) was found. See Methods for further details.

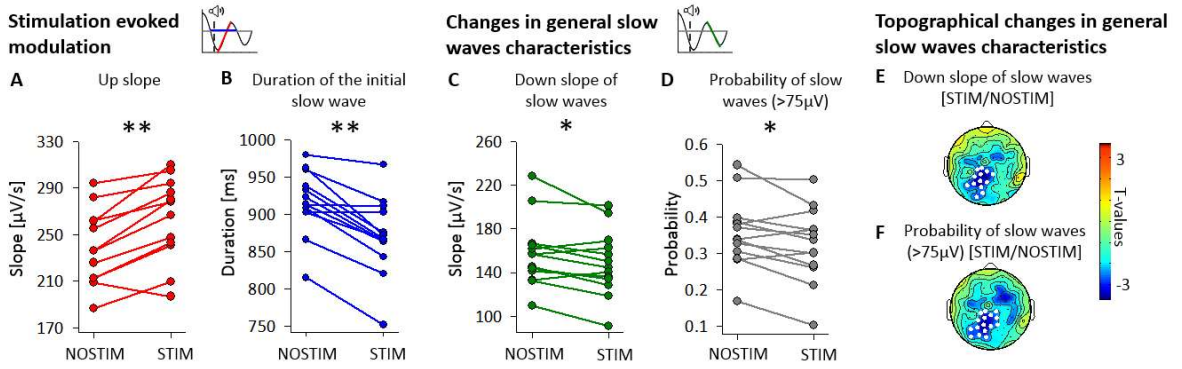

Fig. 3

Stimulation evoked modulation and induced changes on general slow wave characteristics in the hotspot-electrode. (A and B) Stimulation evoked modulation recorded at the hotspot-electrode during the NOSTIM and STIM sessions. The immediate up-slope (red) and duration (blue) of the initial single slow wave (see schema) are shown (mean values of the NOSTIM and STIM session for each subject). * $p < 0.05$, ** $p < 0.01$, paired t-test; n = 13. (C and D) Induced changes in slow wave characteristics during sleep. The down-slope of slow waves (green, mean down-slope) and the probability of slow waves (gray, defined as all waves with a peak-to-peak amplitude larger than 75µV) are shown (mean values of the NOSTIM and STIM session of each subject). * $p < 0.05$, ** $p < 0.01$, paired t-test; n = 13. See Methods for further details. (E-F)Topographical distribution of the statistical comparison (T-values) for changes in general slow wave characteristics. (E) Topographical distribution of the statistical comparison (T-values) for changes of the down-slope of slow waves (mean down slope) between STIM and NOSTIM sessions. (B) Topographical distribution of slow waves probability (defined as all waves with a peak-to-peak amplitude larger than 75µV) between STIM and NOSTIM night. Blue colors indicate a decrease and red colors an increase of the down-slope in the STIM compared to NOSTIM session (paired t-test; n=13, white dots, $p < 0.05$, after nonparametric cluster-based statistical testing).

General sleep architecture was comparable between sessions (Table S1), except for a tendency towards reduced N3 sleep during STIM sleep compared to NOSTIM sleep (NOSTIM: 130.5± 10.6 min; STIM: 117.9±9.8 min; $p = 0.05$; n = 13). Global SWA (mean over all electrodes) did not differ between the two conditions (NOSTIM: 120.1 ± 16.6µV^2/Hz; STIM: 114.4 ± 16.3µV^2/Hz; $p = 0.16$; n = 13). These results confirm that acoustic stimulation *selectively* reduced low-SWA within sensorimotor areas of the trained hemisphere while the general sleep architecture was largely unaffected.

Next we asked whether perturbed SWA disturbs sleep-dependent processes influencing motor learning the next day. During each experimental session neural plasticity was assessed in response to learning a new motor sequence on Mor D1, Eve D1, and Mor D2 (Fig. 1, A and C). Motor learning was quantified by a *Performance Score,* which takes into account the speed-accuracy tradeoff (% correct sequences divided by inter-tap interval in s), and *Tapping Variability* (average standard deviation of inter-tap intervals in completed sequences).

Given that a new sequence was performed during each learning assessment (Mor D1, Eve D1, Mor D2) of each experimental session (STIM, NOSTIM), it is not surprising that a significant increase in Performance Score (Fig. 4A) and a significant reduction in Tapping Variability were observed (Fig. 4B) in both STIM and NOSTIM sessions (training trial effect for each learning assessment and stimulation session, all $p < 0.001$; $n = 11$ after outlier rejection, see Methods and S1). These learning dynamics were highly similar across learning assessments and did not differ significantly between STIM and NOSTIM sessions (all stimulation session × practice trial interactions, $p > 0.91$ for both Performance Score and Tapping Variability; $n = 11$). No effect of STIM versus NOSTIM was observed for the Performance Score (stimulation session × learning assessment interaction, $p = 0.2$; stimulation session main effect, $p = 0.6$; $n = 11$). However, low-SWA perturbation during STIM session sleep significantly increased Tapping Variability on the second morning (Fig. 5B, yellow) in comparison to NOSTIM (Fig. 5B, green; stimulation session × learning assessment interaction $F(2,65.88) = 5.07$, $p < 0.05$; post hoc pairwise analysis, $p < 0.01$; $n = 11$).

Neural changes associated with motor learning were probed by Transcranial Magnetic Stimulation (TMS) and corticomotor excitability was quantified as the peak-to-peak amplitude of motor evoked responses (MEP). We measured excitability as a function of increasing TMS stimulation intensities (input-output curves (IO curves)) which were acquired PRE and POST-training (Fig. 1C) (Rosenkranz et al., 2007a; Zhang et al., 2011). Corticomotor excitability is typically increased (steeper IO curves) after prolonged motor training (Classen et al., 1998; Perez et al., 2007; Rosenkranz et al., 2007a, 2007b; Zhang et al., 2011) indicating LTP-like plasticity (Rosenkranz et al., 2007a).

Learning a new motor sequence in the morning (Mor D1) led to an increase in corticomotor excitability in the first dorsal interosseous (FDI) as shown by a steeper POST-training IO curve. This increase in excitability from PRE to POST learning was observed for both experimental sessions (Fig. 4, C and D; *pre-post* x intensity interaction for STIM and NOSTIM: $F(1,63) \geq 2.97$, $p < 0.05$; $n = 10$). By contrast, corticomotor excitability *decreased* from PRE to POST learning when a new sequence was practiced in the evening (Eve D1) (Fig. 4, C and D; pre-post x interaction effect for NOSTIM: $F(1,63) = 4.18$, $p = 0.01$ and STIM: $p = 0.163$; $n = 10$). Importantly, the neural response to practicing a new sequence in the morning of day 2 (Mor D2) exhibited again an increase following one night of unperturbed sleep (NOSTIM; Fig. 4C; even though the pre-post x intensity interaction failed to reach significance $F(1,63) = 1.26$, $p = 0.29$) while even a slight decrease in excitability on Mor D2 was observed after perturbed sleep (Fig. 4D; $p = 0.99$).

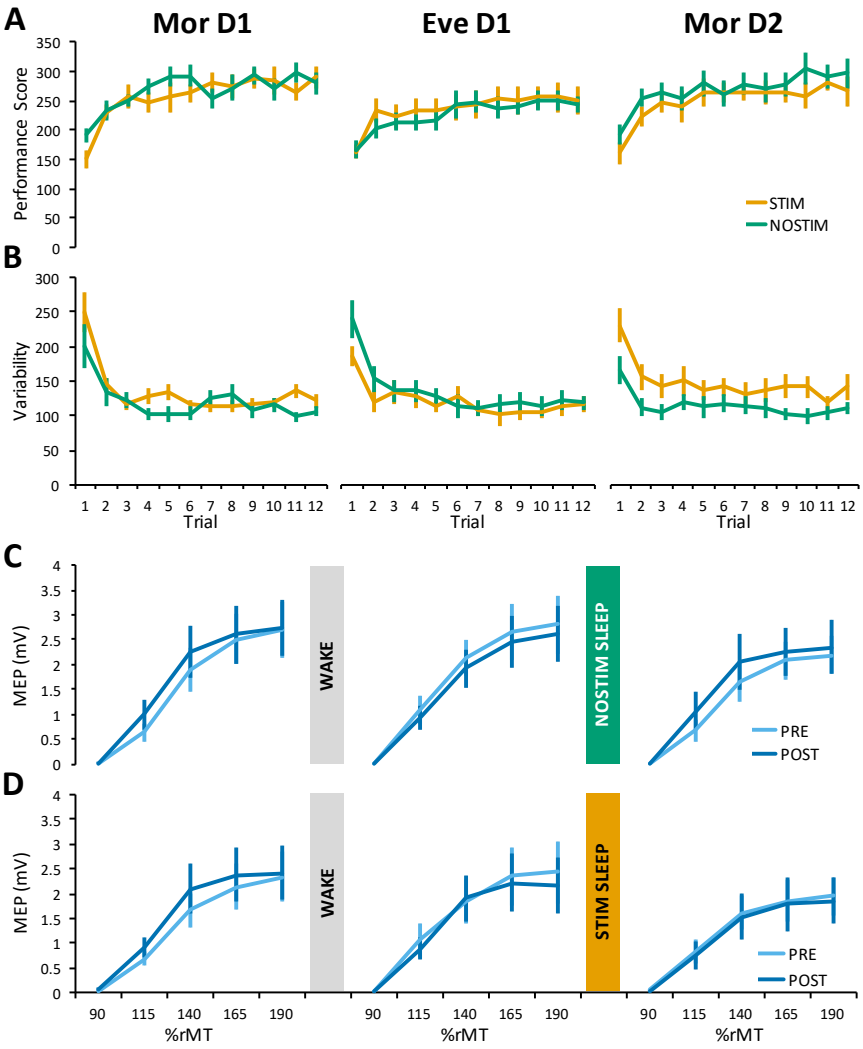

Fig. 4

Behavioral and neurophysiological markers of neuroplastic changes in response to motor training. Data (mean ± SEM) obtained for each learning assessment (Mor D1, Eve D1, Mor D2) are shown for both the NOSTIM session (green) and the STIM session (yellow) as separate learning curves (A and B) and IO curves for the FDI (C and D, see Fig. S6 for other intrinsic hand muscles (see Methods for further details).

Since the steepness of the IO curve can vary strongly across individuals, we summarized the above changes in IO curves by calculating a Facilitation Index for each learning assessment and experimental session which can then be related to SWA: FacIndex = ∫Intensity 1-5MEPpost / ∫Intensity 1-5MEPpre. The FacIndex reflects the normalized change in corticomotor excitability from PRE to POST training and allows us to pool data across the index finger (FDI) and the little finger (abductor digiti minimi (ADM)), i.e. two main muscles involved in the motor learning task (Fig. S6 shows each muscle separately). We observed that the FacIndex was highest on the first morning and decreased in the evening for both STIM and NOSTIM sessions. However, on the morning of day 2, the FacIndex was only restored after unperturbed sleep, while restoration was markedly reduced after perturbing low-SWA in M1 (Fig. 6C*; stimulation session* × *learning assessment* interaction $F(2,37.01) = 3.88$, $p < 0.01$; post hoc pairwise

analysis, $p < 0.01$; n = 10). Additional analyses revealed that these differences between STIM and NOSTIM cannot be explained by changes in baseline corticomotor excitability as measured during PRE, but rather indicate a diminished capacity of plasticity in response to motor training.

Finally, we investigated whether differences in low-SWA measured under the hotspot-electrode between the STIM and NOSTIM sessions (expressed as SWA Ratio = SWAhotspot STIM / SWAhotspot NOSTIM) were related to the degree of overnight changes in either motor variability or corticomotor excitability. We first calculated overnight changes in motor variability (Variability Ratio = VarMorD2 / VarEveD1) and corticomotor excitability (FacIndex Ratio = FIMorD2 / FIEveD1) (Fig. 5, B and C). Then we quantified how much these ratios differed between the STIM and NOSTIM sessions (VariabilityΔRatio, FacIndexΔRatio) and expressed this as a percentage (Fig. 5, E and F).

Interestingly, the SWA Ratio at the hotspot-electrode was positively correlated with the VariabilityΔRatio (Fig. 5H; Spearman's *rho* = 0.71, $p < 0.05$; n = 10 after outlier rejection, see Methods and S1) indicating that subjects whose SWA was strongly reduced during STIM sleep exhibited elevated levels of motor variability the next morning. Moreover, the SWA Ratio at the hotspot-electrode was negatively correlated with the FacIndexΔRatio (Fig. 5I; Spearman's rho = -0.7, $p < 0.05$; n = 9 after outlier rejection, see Methods and S1) indicating that subjects whose SWA was strongly reduced during STIM sleep did not recover their capacity to exhibit a training-induced increase in corticomotor excitability overnight. No correlation was found between the VariabilityΔRatio and FacIndexΔRatio (Fig. 5G; $p > 0.05$) indicating that behavioral markers of motor learning and neurophysiological markers estimating changes of synaptic strength within M1 circuitry in response to learning are statistically independent phenomena. The lack of a direct association between these markers has been demonstrated before (Bestmann and Krakauer, 2015; Carson et al., 2016; Liepert et al., 1999; Ruddy et al., 2016) corroborating the view that changes in corticomotor excitability and behavioral markers of motor learning are two complementary measurements. While behavioral markers are a compound measurement capturing adaptation at multiple levels of the neuromuscular system, corticomotor excitability reflects a subset of the circuits involved in learning with a strong focus on the primary motor cortex (Bestmann and Krakauer, 2015). Importantly, our findings indicate that both markers of learning are significantly modulated by SWA.

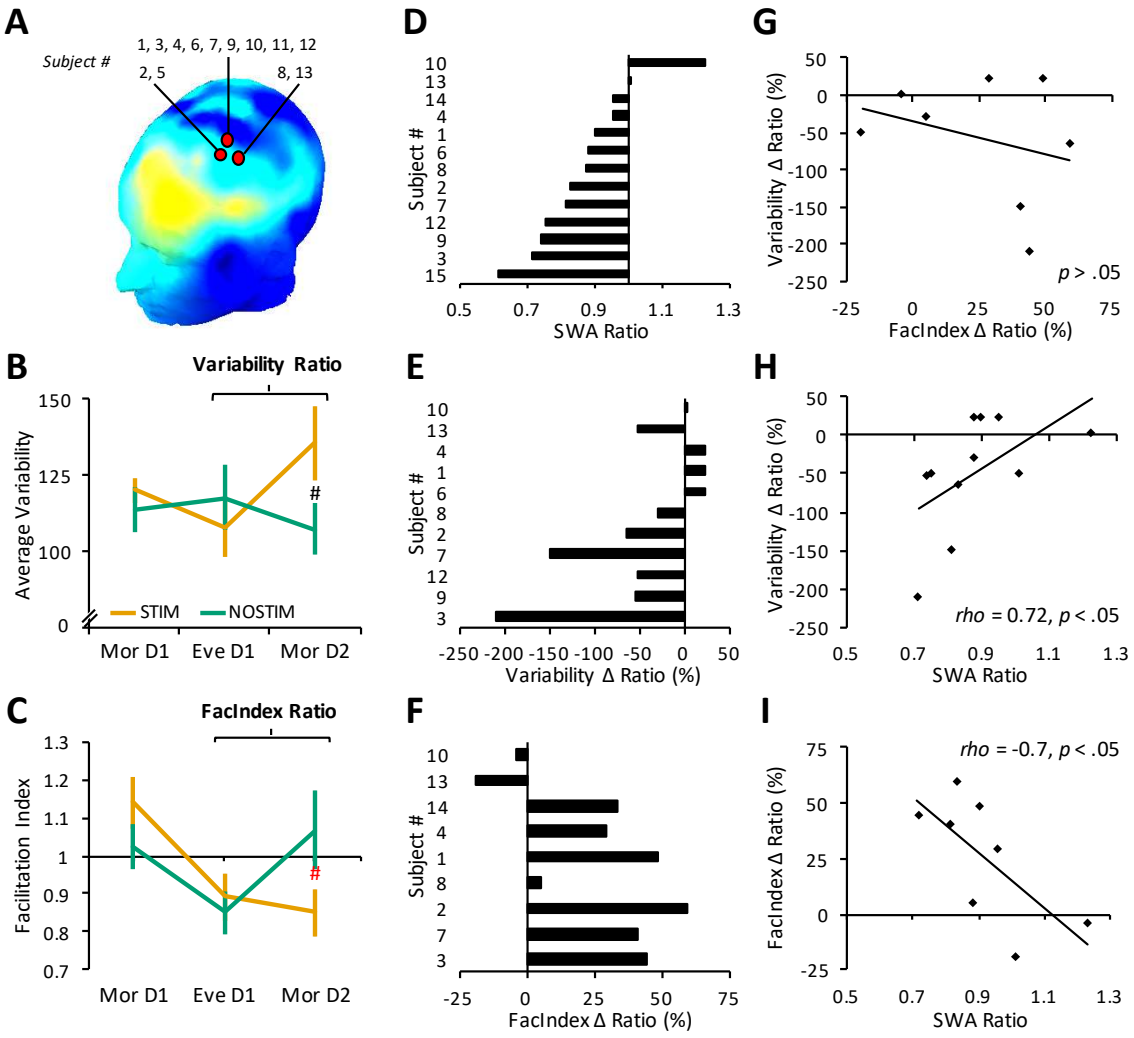

Fig. 5

Correlation analysis between SWA and markers of neuroplastic changes in response to motor training. (A) Hotspot-electrode locations for each subject depicted on top of the topographical map showing low-SWA differences between the STIM and the NOSTIM sessions (blue colors indicate less SWA during STIM sleep; n = 13; see Methods). (B) Plateau performance of Tapping Variability (n = 11) for each learning assessment (Mor D1, Eve D1, Mor D2) in both the STIM and NOSTIM session. (C) Changes in corticomotor excitability (n = 10) for each learning assessment were summarized by a Facilitation Index. A FacIndex > 1 indicates an increase in corticomotor excitability from PRE to POST (see Fig. 4, C and D), while a FacIndex < 1 indicates a decrease. (D) Rank ordered SWA ratio (SWA STIM / SWA NOSTIM) calculated for the hotspot-electrode of all subjects included in the correlation analysis. Note that small SWA ratios (< 1) indicate that less SWA was observed in the STIM than in the NOSTIM session. (E) Overnight change in Variability was calculated for all subjects included in the correlation analysis. Note that a Variability Δ Ratio < 0 indicates an overnight increase in Variability in the STIM compared to NOSTIM session. (F) The perturbation related effect on the overnight change in FacIndex was calculated for all subjects included in the correlation analysis. Note that a higher FacIndex Δ Ratio indicates a loss in the capacity to exhibit a training-induced increase of corticomotor excitability in the STIM session compared to NOSTIM session. (G) There was no significant correlation between Variability Δ Ratio and FacIndex Δ Ratio (p > 0.05). (H) The SWA Ratio and Variability Δ Ratio exhibited a significant positive correlation indicating that a large reduction of SWA during the STIM night was associated with a larger overnight increase in variability when

compared to the NOSTIM session. (I) The SWA Ratio and FacIndex Δ Ratio exhibited a significant negative correlation indicating that a large reduction of SWA during the STIM night was associated with smaller increases in corticomotor excitability in response to motor learning. # post hoc analysis STIM vs NOSTIM ($p < 0.05$); vertical bars denote SEs. See Methods for further details.

Finally, we conducted a control experiment where subjects (n=7) followed the exact same overall procedures but a control electrode overlying right temporo-parietal cortex was targeted during the STIM night. This caused a significant reduction of SWA by 14.46 ± 4.28% in the right hemisphere (Figure 7A,B) while SWA was virtually unchanged at the hotspot electrode of the left hemisphere (increase of 2.74 ± 5.2%). Thus, applying acoustic stimulation to a control electrode in right temporo-parietal cortex modulated SWA at the hotspot electrode significantly less than targeting the hotspot electrode directly. This was quantified by SWA Ratios which did not differ-significantly from 1 and were significantly smaller than when SWA was perturbed at the hotspot electrode (Wilcoxon Ranksum, one-sided, $p< 0.05$, Figure 6C). Also in the control experiment, a significant increase in Performance Score and a significant reduction in Tapping Variability were observed at all time points. However, mean VariabilityΔRatio did not differ significantly from zero (Wilcoxon Sign Rank, $p=0.218$) indicating that perturbing SWA over the right temporo-parietal cortex did not cause an overnight-change in motor variability. In contrast perturbing SWA at the hotspot electrode (main experiment) caused the VariabilityΔRatio to significantly deviate from 0 (Wilcoxon Sign Rank, $p<0.05$). Comparing the VariabilityΔRatio directly between the main and the control experiment failed to reach significance (Wilcoxon Ranksum one-sided, $p=0.218$), however, perturbing SWA at the hotspot electrode caused a behavioural effect (Cohen's $d=0.66$) approximately twice as large than perturbing SWA in right temporo-parietal cortex (Cohen's $d=0.33$). We also analyzed the FacIndex for the same muscles as in the main experiment. We scrutinized in particular whether the STIM versus NOSTIM night changed the FacIndex from the evening of day 1 to the morning of day 2 as indicated by the FacIndexΔRatio, which did not differ significantly from zero (Wilcoxon Sign Rank $p=0.81$), and exhibited a very small effect size (Cohen's $d=0.03$). By contrast, perturbing SWA at the hotspot electrode had a large effect on FacIndexΔRatio (Cohen's $d=1.09$), which differed significantly from zero (Wilcoxon Sign Rank $p=0.027$). Comparing the FacIndexΔRatios of the main and control experiments directly, the result was verging upon significance (Wilcoxon Ranksum one-sided, $p=0.057$). Taken together, the results suggest that perturbing SWA under the hotspot electrode (main experiment) influenced motor plasticity in response to motor training the next morning substantially more than perturbing SWA under the right temporo-parietal cortex (control experiment).

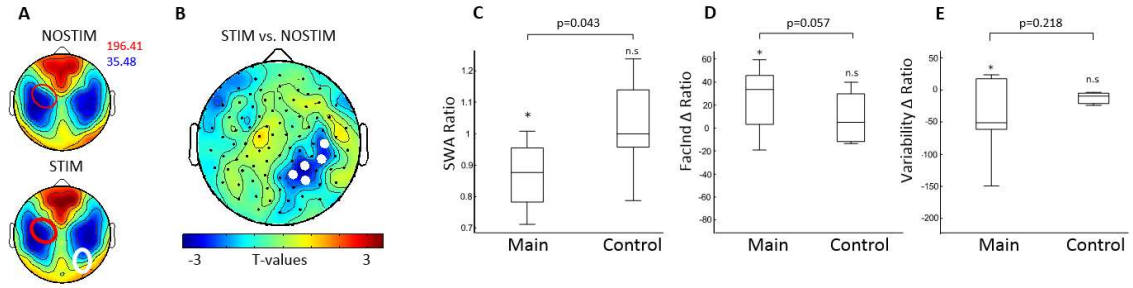

Fig. 6

Control experiment targeting right temporo-parietal cortex (white circle) during STIM night (n=7) (A) Topographical map of low-SWA (1-2Hz) of the two experimental nights scaled to maximum (red) and minimum (blue) power values (μV/Hz²). The red circle indicates the location of the individuals' TMS hotspot which clearly differed from the electrodes selected for local slow wave perturbation (white circle) (B) Statistical comparison (t-values) of low-SWA at the hotspot electrode (red circle) between the STIM and NOSTIM sessions (paired t-test; n =7). (C) SWA Ratio over the hotspot electrode (red circle in A,B) for the main and control experiment. Comparison of the FacIndex Δ Ratio (D) and the VarDeltaRatio (E) between the main experiment and control experiment. * <0.05, Wilcoxon Sign Rank test. See Methods for further details.

Discussion

Using a novel approach to perturb NREM sleep we demonstrated that a reduction of SWA in M1 causally influenced the capacity to undergo motor learning and adaptive brain responses to motor training the next morning, such that larger reductions in SWA due to our experimental manipulation were associated with less efficient adaption at the behavioural and neural level. Interestingly, perturbing SWA in M1 had little influence on average performance gains in response to training, however, it significantly increased tapping variability which remained elevated after reaching plateau performance and it prevented a training-induced increase in corticomotor excitability that is otherwise observed in response to motor training.

This finding in healthy human volunteers was revealed using a non-invasive approach with the defining feature that acoustic stimulation was triggered by slow waves detected by the EEG electrode that was closest to the FDI hotspot, timed such that the slow waves returned more rapidly to the positive up-phase when compared to unperturbed sleep (Fig. 3, A and B). As a consequence large amplitude slow waves occurred less frequently (Fig. 3D) and this effect was only observed for electrodes close to the target area, with a tendency to spread to the back of the head. Since slow waves are not phase locked across different brain areas (Massimini et al., 2004), our paradigm caused these specific local effects because slow waves in M1 were consistently stimulated during the negative phase, while slow waves in other areas were most likely stimulated at random phases (Figure S4). Indeed when targeting slow waves at a different brain area (control experiment), we reduced SWA over that particular brain area. However, slow waves are not stationary. The majority originate in the frontal cortex and travel backwards (Massimini et al., 2004). Therefore, we quantified the travelling of slow waves in an exploratory analysis (using the toolbox presented in Mensen et al. 2016). Slow waves, travelling through the hotspot electrode, passed significantly less electrodes located posterior to the hotspot electrode in the STIM night compared to the NOSTIM night. Thus, by interacting with the ongoing slow oscillation at the hotspot electrode the travelling of slow waves might be interrupted, which may explain the reduction of lowSWA posterior from the hotspot electrode. The precise interaction of local slow wave disturbance and the consequences on slow wave traveling need to be elaborated in more detail in future studies. Moreover, the general sleep architecture was very similar between the two sessions, except for a tendency toward reduced N3 sleep during STIM night (mean difference 12.7±5.6 min). However, the difference in N3 sleep does not seem to be related to the impaired recovery process of cortical excitability nor the reduced improvement in variability (VAR: Spearman's RHO=0.1, p=0.78, FacInd: Spearmans's RHO=-0.17, p=0.68). In addition, psychological measurements (i.e. motivation, mood, attention etc. assessed by questionnaires) were also very similar across sessions, illustrating that the perturbation was highly specific to low-SWA in M1 (see supplementary discussion) and that our behavioural and neural measurements were unlikely confounded by lack of attention, motivation or other side effects typically associated with general sleep deprivation. Of course, our viewpoint from the surface of the scalp provides only an indirect measure of brain activity, as we detect only large population neuronal oscillatory activity. Nevertheless, using this completely non-invasive method we provide the first evidence that *local* deprivation of sleep slow waves can be achieved in humans. The value of this tool is further increased by the recent observation that a similar approach also serves to boost slow waves

(Ngo et al., 2013). As such, this simple and non-invasive approach allowing long-term stimulation is also ideally suited for clinical translation, for example, benefitting new therapeutic approaches that take advantage of sleep to modulate specifically affected brain areas in a way that will not affect daytime performance.

Motor training was tested using different motor sequences because this task is well-suited to repeatedly probe the effect of motor training (de Beukelaar et al., 2016). During all learning assessments motor sequence production was optimized by repetitive training, which resulted in more accurate (Fig. 4A) and less variable motor execution (Fig. 4B). Even though this finding might be surprising at first, one has to keep in mind that we changed SWA in M1 only by approximately 13%. Thus, one would expect that our experimental manipulation causes relatively small behavioral effects which might be most strongly reflected by temporal aspects of task performance as previously observed by (Lustenberger et al., 2016). We found that perturbing SWA in M1 during sleep resulted in general increase in tapping variability the next morning that was significantly elevated when compared to performance after unperturbed sleep (Fig. 4B and 5B). Thus, even though the subjects improved their mean performance, motor execution was generally less efficient. The benefit of local deep sleep for motor behavior may be to keep baseline motor variability low to assure optimality of motor control and learning (Todorov and Jordan, 2002), a result in line with previous evidence indicating that sleep reduces variability of both postsynaptic firing and behavior via a down-scaling mechanism (Hill et al., 2008; Shmuelof et al., 2012).

We further demonstrated that sleep modulated neurophysiological responses to motor training. Motor training has been shown to strengthen input-output relationships of the activated motor circuits (i.e. the same TMS input evokes a larger physiological response after training) most likely by potentiating synapses via a LTP-like mechanism (Butefisch et al., 2000; Rioult-Pedotti, 2000; Rioult-Pedotti et al., 1998). We show that the brain's capacity to exhibit synaptic potentiation is high in the morning but significantly reduced in the evening (Fig. 4C,D; Fig. 5C), lending further support to the hypothesis that synaptic plasticity gets saturated during long periods of wakefulness (de Beukelaar et al., 2016; Vyazovskiy and Harris, 2013). One night of unperturbed sleep increased the brain's capacity to exhibit synaptic potentiation the next morning, however, locally perturbing SWA in motor cortex was sufficient to diminish this effect (Fig. 5C,I). A control experiment further confirmed that overnight changes in the ability to effectively reduce motor variability due to practice as well as changes in the neurophysiological response to motor training were much less influenced when SWA was perturbed in a control region (i.e. right temporo-parietal cortex). Thus, local deep sleep appears to be mechanistically involved in downscaling synaptic potentiation overnight, thus ensuring that the brain's capacity to efficiently adapt to the environment via plastic changes at the synaptic level is restored on a day-to-day basis.

As such we shed light upon important concepts regarding the biological function of sleep and provide the first evidence that there is a causal link between SWA while sleeping and downscaling neural processes that restore the *capacity of plasticity* in response to motor training. Taken together our findings consistently indicate that local deep sleep is essential for maintaining the brain's capacity to respond *efficiently* to motor training and thus for adapting to the environment.

Materials and Methods

Subjects participating in the main experiment

31 naive (no prior experience with the motor task, no musicians) healthy right-handed (96.7 ± 2.4%; Oldfield 1971) subjects (13 females, mean ± SEM age; 22.02 ± 0.34 years) complied with the following inclusion criteria and participated in the main experiment:

no personal or family history of psychopathology, no severe brain injury, no sleep disorders, no chronic diseases, no current use of psychoactive agents or other medications, no travelling across more than 1 time zone in the 4 months before the study, no previous adverse reactions to Transcranial Magnetic Stimulation (TMS), a resting motor threshold (rMT) exceeding 50% of the stimulator output (as tested during a familiarization session, see below). The latter criterion was necessary to protect the TMS equipment from over-heating since our procedure required repeated stimulations of up to 190% rMT. Written informed consent was obtained prior to participation. The study was approved by the local ethics committee and performed according to the Declaration of Helsinki.

Of the 31 subjects that complied with the inclusion criteria, 16 dropped out for the following reasons: discomfort caused by TMS (n=1), poor sleep during screening night (n=9), not sticking to a regular sleep wake cycle or not tolerating the auditory stimulation (n=6; for flow chart of subject dropouts see Figure S1, upper part).

Behavioral task

Subjects performed a computerized six-element finger sequence tapping task (presented with E-Prime; Psychology Software Tools, inc – Sharpsburg, US) adapted from Karni et al (Karni et al., 1998). The sequence to be executed was depicted on top of the laptop screen using a numbering system, with 1, 2, 3, and 4 corresponding to the index, middle, ring and little fingers of the right hand respectively (Fig. 1C). Throughout the experiment eight equally difficult sequences were used. None of these sequences were trained twice and the order of sequences used throughout the experiment was randomized across subjects. While tapping the sequence a black dot appeared on the screen below the current number every time the subject pressed a key indicating that a response was recorded without giving any accuracy feedback. When a sequence was completed the screen was refreshed so that the same sequence appeared on top without any black dots present. One training trial consisted of typing the given sequence for 30s as many times as possible followed by a rest period of 30s to prevent fatigue. A training block consisted of 12 consecutive trials.

Electromyography (EMG) and TMS

Focal TMS was applied with a 70mm figure-of-eight coil connected to a Magstim 200 stimulator (Magstim, Whitland, Dyfed UK). The coil was positioned over the M1 of the left hemisphere, tangential to the scalp with the handle pointing backwards and laterally at 45° away from the mid-sagittal line (Pascual-Leone et al., 2002). The optimal scalp position (“hotspot”) for stimulating the right first dorsal interosseous (FDI) was identified and marked for each learning assessment. The rest motor threshold

(rMT, lowest stimulus intensity evoking MEPs with amplitudes of at least 50μV in 5 out of 10 consecutive stimuli) was determined prioritizing the FDI (Rossini et al., 1994).

EMG data was recorded from FDI, abductor digiti minimi (ADM) and opponens pollicis (OP) of the right hand (Bagnoli, Delsys Inc., Natick, USA) (for an overview of detailed results for all three muscles see Fig. S6). The signals were sampled at 5000Hz (CED Power 1401, Cambridge Electronic Design, UK), amplified, band-pass filtered (5-1000Hz), and stored on a PC for offline analysis. Pre-stimulus EMG recordings were used to assess the presence of unwanted background EMG activity in the 110 to 10ms time interval preceding the magnetic pulse.

Corticomotor excitability was quantified by measuring input-output curves (IO curve) using 90%, 115%, 140%, 165% and 190% of rMT. One IO curve consisted of 20 MEPs per intensity. They were acquired in 2 blocks of 50 MEPs so that per block 10 stimulations were acquired for each of the 5 intensities. In between blocks a rest period of approximately 30s was provided. Within one block the inter-stimulation interval varied between 6 and 7s resulting in a total block time of 5min.

High-density sleep electroencephalography (EEG)

All night sleep was recorded to the vertex (Cz), sampled at 500Hz (0.01-200Hz), using high density (hd) EEG (Electrical Geodesics Sensor Net for long-term monitoring, 128 channels, Electrical Geodesics Inc., EGI, Eugene, OR, US). Electrooculographic (EOG) and submental EMG recordings were acquired for visual scoring of sleep. Two additional electrodes (gold, Grass Technologies, West Warwick, RI, USA) were attached to the earlobes, which served as reference electrodes for the online slow wave detection (see 'SWA topography and sleep slow waves analysis'). After adjusting the net to the vertex and the mastoids, all electrodes were filled with an electrolyte gel to ensure the maintenance of good signals throughout the night. In general, electrode impedances were below 50kΩ. Impedances were below 20kΩ for the electrodes used for online slow wave detection; i.e. submental electrodes, electrodes on the earlobes, and the hotspot-electrode defined as the EEG electrode closest to the scalp position of the FDI hotspot as previously determined by TMS.

Real time closed-loop slow wave detection

Using a custom LabVIEW (National Instruments, Austin, TX, US) program, a closed-loop algorithm detected sleep slow waves in real time (loop time ~30ms) and administered acoustic stimuli to selectively modulate sleep slow waves based on the EEG electrode that was closest to the FDI defined as TMS hotspot electrode as determined by TMS (Fig. 1, B and C). For all subjects the TMS-hotspot electrode was one of the following channels: CH29, CH30 or CH36 within the EEG net. The EEG signal of the TMS hotspot electrode was re-referenced to the mean value of the earlobe electrodes and the signal was band-pass filtered (Butterworth 0.5-2Hz, stop-band <0.1 and >10Hz, stopband attenuation 20dB, passband attenuation 0.1dB). In parallel the submental EMG was monitored, by continuously calculating the root mean square over 2 seconds. Every time the loop was turned on, tones (pink $1/f$ noise of ~50dB) were played for precisely 50ms whenever the EEG signal crossed a default threshold (for the main experiment -30μV, for the control experiment -25, for further detail see: paragraph "control

experiment") and the EMG was below a given threshold that was continuously monitored and adapted by the experimenter. The EMG threshold was implemented as a safety component to prevent stimulation while there were any sign of arousal. The manual stimulation procedure conducted by the experimenter was the following: Stimulation started after 10-15min of stable N3 sleep. The online slow wave detection algorithm was turned on through the night but only during stable NREM sleep stages 2 and 3 (appearance of spindles, K-complexes and slow waves). The goal was to stimulate as much as possible without waking the subjects (on average 3143.69±340.82 stimuli were applied, Table S1). It is important to mention that the overall goal of the study was only to locally disturb slow wave sleep, without interfering with the global structure of sleep. Therefore the detection algorithm was turned off during each transition and after every sign of arousal (i.e. increase in EMG or break down of the EEG) and kept off until the EEG displayed stable N2 or N3 sleep again. As a consequence tones were applied to only ~50% of all slow waves. Offline verification of the online slow wave detection algorithm was performed by measuring the instantaneous phase of each slow wave at tone onset. Therefore the EEG signal of the hotspot electrode was re-referenced to the mean signal from the two earlobes and band-passed filtered between 0.5-2 Hz (using zero-phase infinite impulse response Butterworth filter with the same filter settings as for online slow wave detection, stop-band < 0.1 and > 10Hz), followed by a Hilbert transform to define instantaneous phase. Phase angles (0°-360°) were defined with 0° corresponding to the negative peak of the slow wave cycle. Percentage of tones applied during the negative phase of the slow wave cycle (between 270°-360° corresponding to the first negative half wave or between 0°-90° corresponding to the second negative half wave), were counted.

Overall experimental protocol of the main experiment

Subjects participated in 3 sessions: (i) a screening and familiarization session for TMS and sleeping with an EEG net in the laboratory; (ii) a first experimental session; and (iii) a second experimental session one week later.

TMS screening and familiarization session

After the subject was screened for TMS exclusion criteria and possible adverse effects by the experimenter, he/she was seated in a comfortable chair with the right forearm resting in a neutral position while the hotspot and rMT of the right FDI were determined (see 'EMG and TMS'). Subjects with a rMT exceeding 50% maximal stimulator output were excluded from further participation since the study design required stimulations up to 190% rMT. For subjects with a sufficiently low rMT, one IO curve was obtained in order to familiarize subjects with this measurement technique. Subjects that indicated discomfort during the measurement or reported particular after-effects of the stimulation (e.g. headache) were excluded from further participation. After the corticomotor excitability measurement, subjects sat in front of a laptop on which they performed the behavioral task as it would appear during the experimental sessions. They were instructed to tap a given six-element sequence, i.e. either FAM1 or FAM2 (balanced amongst subjects) as quickly and accurately as possible for 12 training trials of 30s (see 'Behavioral task'). Note that sequences used during familiarization were not re-used during any of the experimental sessions.

Sleep screening and familiarization session

Prior to the experimental session, subjects spent one night in the sleep laboratory of the University Children's Hospital Zurich (Zurich, CH) wearing the hd-EEG net in order to adjust to the experimental environment and to exclude possible sleep disorders. Sleep quality was assessed, and only subjects with a sleep efficiency of at least 80% were included in the experiment.

Experimental sessions

After successfully participating in both TMS and EEG screening sessions, subjects were instructed to keep a constant sleep schedule for ~7 days (range from 4-8 days) preceding the first experimental session. Compliance to the schedule was assessed using both daily sleep diaries and wrist actigraphy (Actiwatch Type AWL from Cambridge Neurotechnology, CamNtech, Cambridge, UK or Geneactiv). Subjects were required to refrain from alcohol and medication 48h prior to each experimental session. During the experimental sessions, subjects did not perform strenuous exercise, and were not allowed to nap during the day (as instructed and verified via self-report and actigraphy). Women were tested during the follicular phase of their menstruation cycle to prevent the variation of sleep EEG activity markers as a consequence of hormonal fluctuations (Driver et al., 1996).

Subjects were tested in two experimental sessions that were separated by 1 week but had an identical chronological setup of three separate learning assessments; the first in the morning of day 1 (Mor D1), the second in the evening on that same day (Eve D1), and a third in the morning the next day (Mor D2, Fig. 1A). In one of the two experimental sessions, slow waves were perturbed using acoustic stimulation (STIM) precisely time-locked to the down-phase of sleep slow waves detected in the TMS hotspot-electrode (Fig. 1B). In the other experimental session subjects heard no tones (NOSTIM). The order of the STIM versus NOSTIM experimental session was counterbalanced among subjects (6 subjects started with STIM).

The first learning assessment started (approximately) 30 min after waking in the morning and subjects filled out a questionnaire rating the sleep quality of the night before (i.e. assessing subjective sleep score with a visual analog scale) and a questionnaire rating psychological measurement (i.e. attention, mood, concentration and motivation, also on a visual analog scale, which was assessed before each learning session, i.e. in the evening and the next morning). The hotspot and rMT for the right FDI were then determined and corticomotor excitability was measured via an IO curve (PRE). Subsequently subjects performed motor training, i.e. they practiced a new six-element motor sequence (e.g. sequence A) for 12 trials (i.e. 30s tapping followed by 30s rest) lasting 12min in total. Corticomotor excitability was re-measured immediately afterwards by a second IO curve (POST) in order to determine changes indicative of motor plasticity as a consequence of training (Fig. 1C). It has been shown that repetitive motor training leads to an increase of corticomotor excitability, an effect that is NMDA receptor dependent, sensitive to GABAergic inhibition (Butefisch et al., 2000), and it occludes subsequent experimental induction of long-term-potentiation (Kirkwood et al., 1996; Rosenkranz et al., 2007a; Stefan et al., 2006). Together these findings strongly suggest that increases of corticomotor excitability in response to motor training reflect, at least partly, a LTP-like mechanism at the synaptic level activated

by extensive motor practice and associated motor learning (Butefisch et al., 2000; Rosenkranz et al., 2007a; Stefan et al., 2006).

The second learning assessment on the evening of the same day started ~2h 30min before bedtime. Subjects underwent an identical procedure as during the morning, with the only difference being a novel finger sequence was trained (e.g. sequence B). Immediately afterwards the subjects were prepared for EEG measurements. Before subjects went to bed they were asked to perform a short attention task, based on an auditory oddball paradigm. During the task, participants had to sit quiet in front of a screen and fixate upon a white cross on a black background. Over a period of ~6 minutes participants listened to 450 stimuli with an inter-stimuli interval of 0.8 seconds, whereof 90% were standard tones (880 Hz) and 20% were deviant tones (988 Hz, presented in a random order). Subjects were asked to react as fast as possible to the deviant tones with a mouse click. Reaction time to the deviant tones, number of missed clicks and number of wrong clicks were recorded as performance scores. After the attention task subjects went to bed at their usual bedtime. Sleep episodes were scheduled individually according to the subject's reported bed times with a sleep time of ~8h.

On the morning of the next day, subjects were awakened by the experimenter. After performing the same attention task as in the evening the EEG net was removed and a shower was taken within 30min before starting the third learning assessment. This test was timed so that learning the novel sequence (e.g. sequence C) took place 24h after acquiring the first sequence on the morning of the first day. Identical to the previous learning assessment performed on the previous morning, subjects filled out a questionnaire regarding their quality of sleep the night before and the questionnaire rating psychological measurement, after which corticomotor excitability was tested PRE and POST sequence tapping.

This complete experimental session combined three learning assessments that spanned over 2 consecutive days including one night in the sleep laboratory. It was repeated 7 days later and subjects were therefore instructed to keep the same constant sleep schedule for the following 7 days preceding the second experimental session, which was again assessed using both daily sleep diaries and wrist actigraphy. The second experimental session followed an identical protocol to the first one with the only difference being the sleep slow wave intervention (i.e. STIM versus NOSTIM) and that three new sequences were learned (e.g. sequences D, E, F) during the three learning assessments.
Assignment of sequences to experimental conditions and learning assessments was randomized and counter balanced among subjects.

Outlier detection and removal for the main Experiment

We aimed for a good balance between keeping the sample size for each analysis as high as possible whilst applying a stringent outlier detection strategy which we defined a-priori and applied consistently to all parameters. Outlier detection and removal is particularly important to minimize the risk of bias due to uncontrolled confounders. For example, we were very strict to include only participants that had high-quality sleep in both the STIM and the NOSTIM night. Otherwise our data might have been confounded by large differences in the general sleep architecture. For each parameter, we calculated the mean and

standard deviation across all participants available for the specific comparison (i.e. with potential outliers included in the dataset). An individual's data were identified as outlier when it fell outside of the mean ± 2* stdev range and this individual was then removed from the statistical analysis. Outlier detection and removal was performed stepwise as detailed in Figure S1, lower part). The final analyses were performed on the following sample sizes: SWA analysis (n=13), Performance Index and Variability (n=11), correlation between SWA Ratio and Variability Δ Ratio (n=10), FacIndex analysis (n=10), correlation between SWA Ratio and FacIndex Δ Ratio (n=9).

Data analysis and statistics

Inferential statistics were computed using Mixed Effects Models in SPSS (Version 16.0, SPSS Inc. Chicago, US), as they account for covariances between related data samples in repeated measures designs, and have greater flexibility for modeling effects over time than traditional ANOVA approaches (Gueorguieva and Krystal, 2004). Moreover, they use all available data, allowing inferences to be made even from small sample sizes, and they do not assume normality of the raw data (Gelman and Hill, 2006). Fitting of the mixed effects models employed restricted maximum likelihood estimation (REML) and a compound symmetry or unstructured covariance matrix. Model fit indices (Akaike Information Criterion and Schwarz Bayesian Criterion) were considered prior to choosing the covariance matrix and model type. The influence of each of the fixed effects on the model was estimated using F tests. In all models subject was designated as a random effect with random intercepts.

Behavioral task

Key presses were recorded and *accuracy* (%) was calculated as the number of correct sequences divided by all completed sequences during each 30s trial. Performance *speed* was measured as the time (ms) between key presses, i.e. the inter-tap interval (ITI). A *Performance Score* was calculated for each subject and trial by dividing the accuracy percentage by the ITI, with higher scores indicating better performance (see also de Beukelaar et al. 2014). Tapping *Variability* was determined by calculating the standard deviation in ITI per typed sequence and was then averaged for each 30s trial and subject. Plateau performance was estimated by averaging the Performance Score / Variability across trial 9-12, i.e. during the last third of the training block when behavioral changes were minor.

Mixed effects models were performed on both the Performance Score and Variability data with repeated fixed effects of *stimulation session* (STIM, NOSTIM), *learning assessment* (MorD1, EveD1, MorD2) and *training trial* (1 to 12). Plateau Performance Score and Variability data were analyzed with an analogous model containing the fixed effects *stimulation session* and *learning assessment*.

Corticomotor excitability

Corticomotor excitability was quantified by MEP peak-to-peak amplitude. MEP amplitude is known to be modulated by EMG background activation (Devanne et al., 1997; Hess et al., 1987). Therefore pre-stimulus EMG recordings were used to assess the presence of unwanted background EMG activity in the 110 to 10ms preceding the magnetic pulse and were quantified via root mean square scores (RMS) across this interval. The maximal and minimal MEPs obtained per intensity and per IO curve were

excluded as well as the obtained MEPs preceded by background EMG higher than 0.01mV. For each subject and over all trials we calculated the mean and standard deviations of the background EMG. Background EMG values deviating from the mean by more than 2.5 standard deviations, and MEPs with a peak-to-peak amplitude which exceeded Q3 + 1.5 x (Q3 - Q1) were removed from further analysis, with Q1 denoting the first quartile and Q3 the third quartile computed over the whole set of trials for each subject. Based on these criteria 15.2 ± 2.3% of the collected MEPs were excluded from further analyses. Furthermore we averaged MEP amplitudes for each stimulation intensity of each IO curve that was recorded and these averages where then subjected to group statistics.

The influence of motor training on corticomotor excitability as quantified by the IO curve was analyzed using a mixed effects model with repeated fixed effects *stimulation session* (STIM, NOSTIM), *learning assessment* (MorD1, EveD1, MorD2), *pre-post* (PRE, POST) and *intensity* (90%, 115%, 140%, 165%, 190%). Changes in baseline corticomotor excitability (i.e. PRE IO curve measured prior to motor training) across learning assessments were tested for each of the two experimental sessions by separate mixed effects models with the factors *learning assessment* (MorD1, EveD1, MorD2) and *intensity* (90%, 115%, 140%, 165%, 190%).

Finally we directly compared whether changes in corticomotor excitability induced by motor training differed between sessions and learning assessments. Therefore, we calculated the integral underneath the IO curve measured before and after motor training (Carson et al., 2013), and calculated a facilitation index, *FacIndex* = $\int_{\text{Intensity 1-5}}\text{MEP}_{\text{post}} / \int_{\text{Intensity 1-5}}\text{MEP}_{\text{pre}}$

A FacIndex > 1 indicates that an increase in corticomotor excitability is observed from PRE to POST training, while a FacIndex < 1 represents a decrease. The FacIndex was calculated for the 2 stimulation sessions and for each of the 3 learning assessments and was entered into a mixed effects model with repeated fixed effects *stimulation session* (STIM, NOSTIM) and *learning assessment* (MorD1, EveD1, MorD2).

We also analyzed general non-task related variations in baseline corticomotor excitability from morning to evening as measured at PRE, i.e. prior to any motor practice. Corticomotor excitability at PRE increased significantly from the morning (MEP amplitude mean ± SEM; STIM: 1.35 ± 0.27mV; NOSTIM: 1.53 ± 0.34mV; n = 10) to the evening (MEP amplitude mean ± SEM; STIM: 1.90 ± 0.42mV; NOSTIM: 1.94 ± 0.34mV; *learning assessment* effect F (1,142) = 21.38, $p < 0.001$; n = 10) but decreased again after a night of sleep, irrespective of whether SWA was perturbed or not (MEP amplitude mean ± SEM; STIM: 1.61 ± 0.40mV; NOSTIM: 1.53 ± 0.34mV, *learning assessment* effect F(1,133) = 8.50, $p < 0.05$; n = 10). Thus, sleep generally decreased corticomotor excitability but only unperturbed SWA in M1 restored the ability to increase it again in response to motor training the next day. It is noteworthy to point out that these data indicate a distinction between general circadian morning-evening increases in corticomotor excitability that remained unaffected by perturbation (returning to baseline each morning), and the specific task-related modulation resulting directly from training the motor task.

Slow wave activity (SWA) topography and sleep slow waves analysis

Sleep EEG was band-pass filtered between 0.5 and 50Hz and down sampled to 128Hz. Sleep stages and arousals were visually scored for 20s epochs according to standard criteria (Iber et al., 2007) by a sleep expert, and verified by another sleep expert (both of them were blind to the experimental conditions). After visual and semiautomatic artefact removal (Huber et al., 2000) the remaining data of each subject were re-referenced to an average value across all 109 channels above the ears. In one subject only the first 4h of the night could be analyzed due to poor data quality for the remaining part (subject #2). Spectral analysis (1-25Hz divided into different frequency ranges: low-SWA (1-2Hz), SWA (1-4.5Hz), theta (4.75-7.75Hz), alpha (8-9.75Hz), sigma (10-15Hz) and beta (20-25Hz), for a detailed overview of the results see Figure. S3) of consecutive 20s epochs (FFT routine, Hamming window, averages of five 4s epochs, resolution of 0.25Hz) of each channel was performed and data were log-transformed for parametrical statistical analysis. Comparisons between STIM and NOSTIM sessions were performed for an individual hotspot-electrode (i.e. the EEG electrode showing the strongest reduction of low SWA during STIM night out of the three selected TMS-hotspot electrodes (CH30, CH29 and CH36 of the HCG sensor Net 128 of EGI) to account for anatomical differences related to the combined MEP of ADM and FDI as done previously e.g. in Huber et al. (2004) as well as for each EEG channel.

Further analysis based on single sleep slow waves was performed for the individual hotspot-electrodes. For both sessions, the EEG signal (sampling rate 500Hz) of the hotspot-electrode was offline re-referenced to the mean value of the earlobe and filtered, using the same filter as for the online detection (Butterworth 0.5-2Hz (stop-band < 0.1 and > 10Hz)).In a first step the immediate response to the acoustic stimulation was analyzed. Therefore the following procedure was applied:

(i) For both nights, sleep slow waves were detected offline (similar to the online algorithm applied during STIM session). A trigger was set, every time the signal crossed a default threshold of -30μV, in all artifact free N2 and N3 epochs.

(ii) All detected slow waves slower than 0.5Hz were excluded to ensure that the signal is not influenced by the filter.

(iii) To account for overall changes in slow wave characteristics (in particular the slope; Riedner et al. 2007) we controlled for the number of detected slow waves across the night. To do so, individual sleep cycles were defined for both nights and only sleep cycles including at least 100 triggers during both nights were considered for the analysis.

(iv) For the NOSTIM session the timing of the first trigger was individually matched to the timing of the first trigger of the STIM session (after 10-15min of stable N3 sleep).

(v) For the STIM session only triggers where a tone was presented during the night were included. During the STIM session we aimed to stimulate as much as possible without waking up the subjects. As

a consequence the stimulation algorithm was turned off by the experimenter if there was any sign of arousal, or during the cycle transitions (from NREM to REM or vice versa). Thus, some slow waves were missed during the STIM session, where no tones were played. Hence, less slow waves were detected during the STIM compared to the NOSTIM session. To account for this difference in the number of detected slow waves, we matched the number of analyzed waves between the two nights. To do so, for each sleep cycle, equal amounts of detected slow waves during the STIM session were randomly selected in the NOSTIM session. Next, the immediate peak-to-peak up-slope following the stimulation (dividing peak-to-peak amplitude by the time between negative and positive amplitude) and the duration of the slow waves were calculated (time between the negative zero-crossing (start of the down-phase) prior to stimulation and the next negative zero-crossing (end of the up-phase)). This approach led to similar results as calculating the median peak-to-peak up-slope and duration of slow waves, including all detected slow waves. Since the former approach always results in slightly different numbers, the median peak-to-peak up-slope and duration of slow waves were taken for further statistics.

In a second step the induced changes of slow wave characteristics throughout the night (i.e. not only including slow waves which were directly disturbed) were analyzed. For this the following procedure was applied:

(i) All waves starting from the zero crossing of the up-phase, i.e. the positive part of the wave, and ending at the second zero crossing of the next down-phase of any amplitude between 0.5 and 2Hz during N2 and N3 sleep were detected offline, using a similar routine as described by Riedner et al. (2007).

(ii) From these detected waves the mean peak-to-peak down-slope (dividing peak-to-peak amplitude by the time between positive and negative amplitude) for each subject and night were calculated.

(iii) Moreover, the probability of slow waves (defined as slow waves with a peak-to-peak amplitude > 75μV) was calculated by dividing the number of detected slow waves (>75 μV)) by the total number of detected waves. For exploratory purposes the analysis of induced changes on general slow wave characteristics was extended to all channels (for this analysis data were down sampled to 128Hz to reduce computational load).

Since all sleep data were normally distributed (normality of the data was confirmed using Shapiro-Wilk-Test), paired t-tests (two-tailed) were used for intra-individual comparisons. For topographical comparisons nonparametric cluster-based statistical testing was applied, using a suprathreshold cluster analysis to control for multiple comparisons (Ferrarelli et al., 2007; Huber et al., 2004; Nichols and Holmes, 2002; Ringli et al., 2013). In short: For each topographical statistical analysis (paired Student's t-test and spearman correlation) new datasets were generated by randomly relabeling the condition label from original data and either paired Student's t-test or spearman correlations were performed, respectively. For each permutation the maximal size of resulting clusters with neighboring electrodes reaching a t-value above the critical value was counted to build a cluster size distribution. From this cluster size distribution the 95th percentile was defined as critical cluster size threshold. For the true

comparison (STIM vs. NOSTIM) only electrodes reaching a t-value beyond the CV and located within a cluster larger than the critical cluster size threshold were considered as significant (paired Student's t-test: CV=2.3, number of permutations 5000, n=13, spearman correlations: CV=0.68, number of permutations 1000, n=9 and CV=0.6, number of permutations 1000, n=12). All offline analyses of the sleep data and statistical analyses were performed in MATLAB (Math Works).

Correlation analysis

We calculated a *SWA Ratio* ($SWA_{hotspot}$ STIM / $SWA_{hotspot}$ NOSTIM) representing the change in SWA measured at the hotspot-electrode between the STIM and NOSTIM sessions. Note that a SWA Ratio < 1 indicates reduced SWA at the hotspot-electrode during the STIM compared to the NOSTIM condition. We also calculated a ratio of overnight change in variability (*Variability Ratio*) by dividing the Variability data obtained in the morning on day 2 by the Variability of the evening on day 1 (Var_{MorD2} / Var_{EveD1}). This was conducted separately for the STIM and NOSTIM sessions. Then, the effect of perturbation was quantified by expressing the difference between the STIM and NOSTIM ratio as a percentage of NOSTIM (*Variability Δ Ratio*). This percentage represents the perturbation related effect on the overnight change in Variability.

*VariabilityΔRatio = (Variability Ratio NOSTIM - Variability Ratio STIM) /(Variability Ratio NOSTIM) * 100*

Note that a lower Variability Δ Ratio indicates an increase in Variability in the STIM session compared to the NOSTIM session. Identical calculations were performed on the FacIndex data. We calculated a ratio of overnight change in FacIndex (*FacIndex Ratio*) by dividing the FacIndex calculated for the morning on day 2 by the FacIndex calculated for the evening on day 1 (FI_{MorD2} / FI_{EveD1}). This was conducted separately for the STIM and NOSTIM sessions. Then, the effect of perturbation was quantified by expressing the difference between the STIM and NOSTIM ratio as a percentage of NOSTIM (*FacIndex Δ Ratio*). This percentage represents the perturbation related effect on the overnight change in FacIndex.

*FacIndex Δ Ratio = (FacIndex Ratio NOSTIM - FacIndex Ratio STIM) /(FacIndex Ratio NOSTIM) * 100*

Note that a higher FacIndex Δ Ratio indicates a loss in capacity to exhibit a learning-induced increase in corticomotor excitability in the STIM session compared to the NOSTIM session. To explore whether changes in SWA caused by STIM versus NOSTIM were statistically related to overnight changes in behavioral and neural markers of plasticity, Spearman's rank correlation coefficients were calculated between (i) SWA Ratio and Variability Δ Ratio and (ii) SWA Ratio and FacIndex Δ Ratio.

These analyses used low-SWA (1-2Hz) measured at the hotspot-electrode, however, control analyses were conducted for all other frequency ranges and at all 128 electrodes (fig. S1). The alpha level for all statistical tests was set initially to 0.05 and significant interactions were further analyzed by post hoc tests, corrected for multiple comparisons in line with the Modified Bonferroni procedure (Keppel, 1991), resulting in an adjusted alpha value of 0.03. Data are presented as mean ± SEM.

Control Experiment

14 subjects complied with the inclusion criteria but 7 dropped out due to poor sleep during screening night (n=4), not sticking to a regular sleep wake cycle or not tolerating the auditory stimulation (n=3). Seven naïve, healthy, right-handed subjects (23.76±1.03 years, 4 females) completed the control experiment. The control experiment followed the exact same experimental procedure as the main experiment, only that slow waves were perturbed using acoustic stimulation (STIM) precisely time-locked to the down-phase of sleep slow waves detected at an electrode overlying the temporo-parietal cortex of the right hemisphere (i.e. the hemisphere ipsilateral to the moving hand). Because the mean amplitude of slow waves is generally lower over the temporo-parietal region compared to the TMS hotspot region (i.e. primary motor cortex) we also adapted the threshold for online slow wave detection slightly, to ensure that in the control experiment the same number of slow waves were detected as in the main experiment. To do so we calculated the mean amplitude over the temporo-parietal electrode (control electrode) and the hotspot electrode of the main experiment. We found that the mean amplitude was reduced by ~20% over the temporo-parietal cortex compared to the hotspot electrode. As a consequence we reduced the amplitude threshold from 30μV to 25μV for the control experiment. The electrode over temporo-parietal cortex was selected because the right temporo-parietal area is believed to have only a minor contribution to the sequence learning task and previous experiments from our lab have indicated that coherence in the SWA frequency range was low between the hotspot electrode and control electrode over right temporo-parietal cortex, indicating that slow waves occur independently in the two areas.

Outlier detection, Data analysis and Statistics for the Control Experiment

We calculated the same parameters as above. No outliers were detected when we applied the same cutoff-criteria as in the main experiment. Our statistical analysis focused on the Variability Δ Ratio and the FacIndex Δ Ratio because these parameters summarize how STIM versus NOSTIM influences changes of behavioral and electrophysiological learning indices between the evening of Day 1 and the morning of Day 2. Since the sample size of the control group (n=7) and the sample size of the experimental group (n<=13) was relatively low we applied non-parameteric statistics. We used i) the Wilcoxon Sign Rank test to probe whether STIM induced SWA reduction, Variability Δ Ratio or the FacIndex Δ Ratio differed significantly from zero for either the experimental or the control group; and ii) the Wilcoxon Rank Sum test for probing whether STIM induced SWA reduction, Variability Δ Ratio or the FacIndex Δ Ratio differed significantly between the main and the control experiment. Since we had a clear directional hypothesis the Wilcoxon Rank Sum test was one-sided. Additionally, we calculated Cohen's d effect sizes for each of the above parameters and each group.

Supplementary Materials

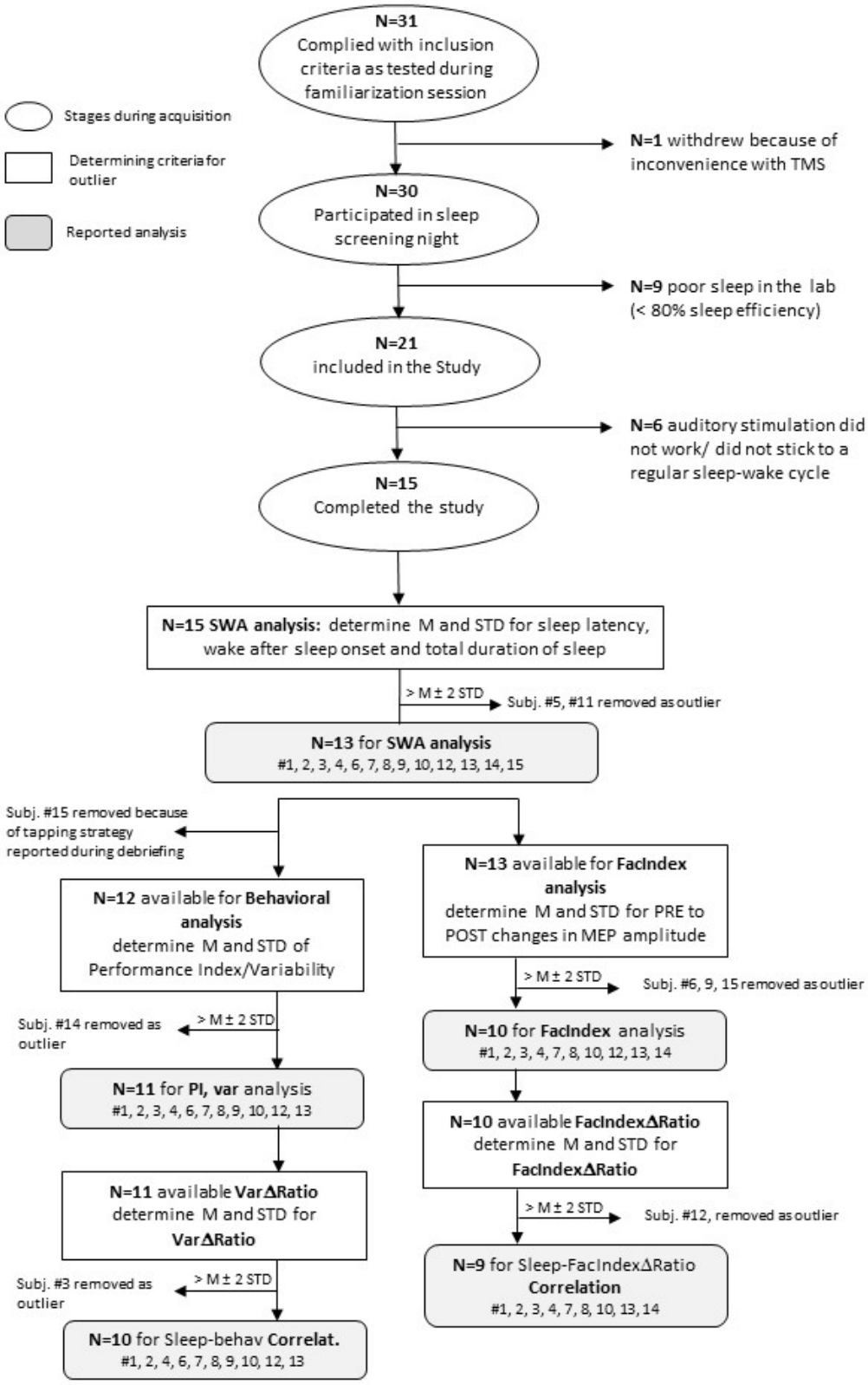

Figure S1:

31 subjects complied with the inclusion criteria as tested during the familiarization session. The upper part of the figure (ovals) indicates how many subjects dropped-out at different stages of the experiment. The lower part indicates how the criteria for detecting outlier were defined (squares) and which participants were included in each of the analyses reported in the manuscript (shaded squares). Outliers were detected by calculating, for each parameter, mean and standard deviation across all participants available for the specific comparison (i.e. with potential outliers included in the dataset). An individual's data were identified as outlier when it fell outside of the mean ± 2* stdev range and this individual was then removed from the statistical analysis. Outlier detection and removal was performed stepwise: We first scrutinized whether sleep quality was comparable between the two nights. This is important because the goal of our study was to disturb slow wave sleep locally while keeping the global sleep structure intact. We chose sleep latency, wake after sleep onset and total duration of sleep as key parameters and calculated the difference between the STIM and the NOSTIM night. In two subjects at least one of these three parameters exceeded the group mean ± 2*stdev and these subjects were removed from further analyses because changes in the general sleep architecture might have a big influence on both behavioral and TMS measurements of motor learning.

For the remaining 13 subjects, we identified outliers with regard to our TMS measurement by assessing changes in mean corticomotor excitability from PRE to POST which were then averaged across stimulation session (STIM, NOSTIM) and learning assessment (MorD1, EveD1, MorD2). We then calculated the mean and stdev of these data across the 13 subjects and found that three subjects had values exceeding the mean + 2 stdev leaving n=10 for the FacIndex analysis.

Next we calculated the FacIndexDelatRatio and detected one outlier which was removed from the dataset leaving n=9 to be included in the correlation analysis testing for associations between differences in SWA at the hotspot electrode and the FacIndexDelatRatio.

For the behavioural data, we had to remove one subject based on the debriefing because he/she reported that he/she tapped a specific rhythm which confounds the variability measurements. After this subject was removed, our outlier detection procedure was applied: each of the behavioral parameters (Performance Index, Tapping Variability) was averaged across trials (1..12), stimulation session (STIM, NOSTIM) and learning assessment (MorD1, EveD1, MorD2). We then calculated the mean and stdev of these data across the 12 subjects and found that one subject's variability measurement exceed the group mean by more than 2 stdev. This subjects was removed from both the performance Index and the Variability analysis leaving n=11. Next we calculated the VarDeltaRatio but had to remove one participant who exceeded the outlier criterion. Thus, n=10 subjects were included in the final correlation analysis testing an association between SWA reductions observed at STIM versus NOSTIM and the VarDeltaRatio.

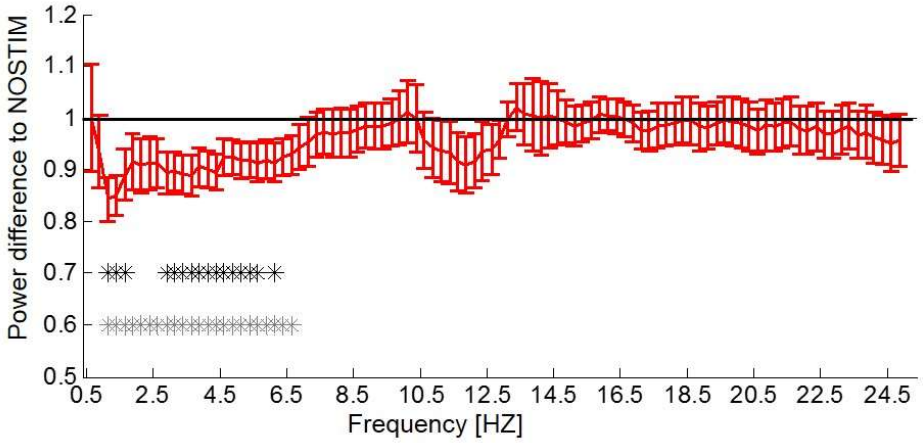

Figure S2

Spectral power (0.5-25Hz, divided in 0.25Hz bins) for the hotspot electrode of the STIM night expressed relative to the NOSTIM night (mean ± sem; 1 = spectrum power of the NOSTIM night, black stars represent p<0.05 and gray stars represent a p<0.1). Note, the strongest reduction was found for frequencies between 1-1.75 Hz, however, also higher frequencies within the delta and theta range (up to 6.75 Hz) show a similar tendency.

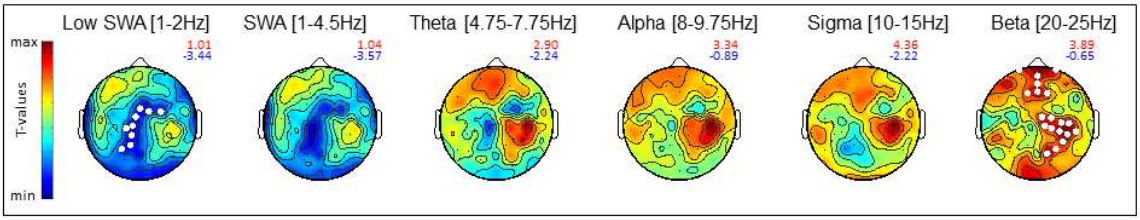

Figure S3

Statistical comparison (t-values) of power values between STIM and NOSTIM sessions for different frequency bands. Blue colors indicate a decrease and red colors an increase in power values in the STIM compared to NOSTIM session. The numbers in the upper right corner indicate the maximum (red) and minimum (blue) t-value for each map. Significant electrodes are marked with white dots ($p < 0.05$, paired t-test; n = 13, after nonparametric cluster-based statistical testing). Note, compared to low-SWA, beta power revealed an increase over frontal and right central/temporal cortices in the STIM session compared to the NOSTIM session. However, it is important to mention that none of these two clusters correlated with the FacIndex Δ Ratio or Variability Δ Ratio (after for a detailed overview of all correlations see Table S2).

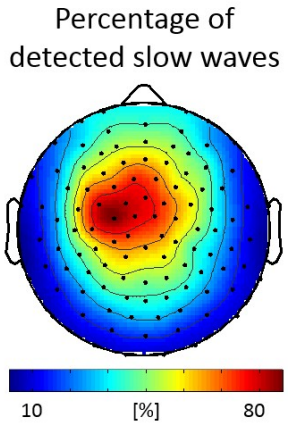

Figure S4

Percentage of slow waves (≤-30µV) detected within a time window of ± 50 ms when a tone was applied for each channel. Note that the fewer slow waves were detected in electrodes further away from the hotspot electrodes.

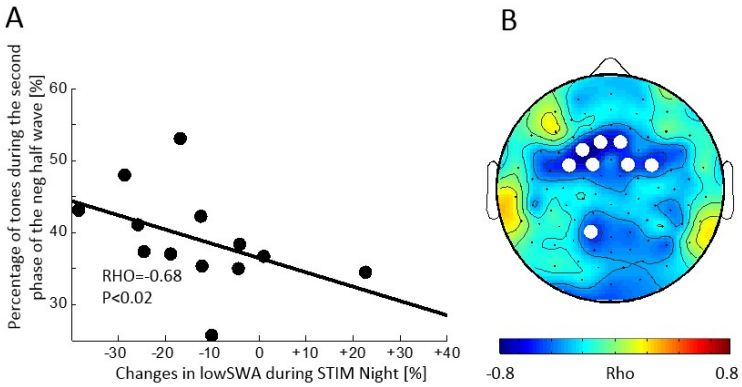

Figure S5

Relationship between phase timing of tone onset at the hotspot electrode and the local reduction in lowSWA in the STIM compared to the NoSTIM night. The phase timing of the online slow wave detection algorithm was quantified offline using Hilbert transformation (based on the signal of the hotspot electrode). On average 82% of all tones were applied during the negative phase of the slow wave cycle. We also perfromed a more detailed analysis of the phase timing by dividing the negative half wave into the first half of the half-wave (i.e. from the first zero crossing to the minimum) and the second half of the negative half wave (i.e. form the minimum to the subsequent zero crossing). To further determine whether phase timing of the tone might contribute to this observed variability in lowSWA changes, the percentage of tones applied during the first versus the second half wave was correlated with the lowSWA changes in the hotspot electrode (Spearman's RHO coefficient) as shown in (A). We found that the more tones were played during the second half of the negative half-wave, the more lowSWA was reduced in the hotspot electrode. (B) Correlation between changes in lowSWA for all channels and the percentage of tones allocated during second half of the negative half-wave (Spearman's RHO coefficient, white dotes $p<0.05$, uncorrected). Note: The correlation was strongest around the hotspot electrode suggesting that the effect of the auditory stimulation was very local.

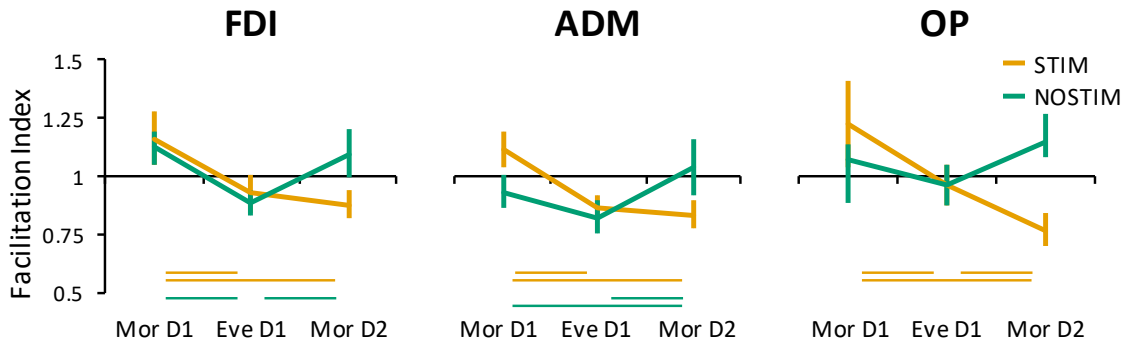

Figure S6

Changes in corticomotor excitability for each learning assessment were summarized by a Facilitation Index (FacIndex = ∫Intensity 1-5MEPpost / ∫Intensity 1-5MEPpre) and are shown for each muscle (FDI, ADM and OP). A FacIndex > 1 indicates an increase in corticomotor excitability from PRE to POST training, while a FacIndex < 1 indicates a decrease. For each muscle, a stimulation session × learning assessment interaction was found ($F \geq 3.88$; $p \leq 0.03$; $n = 8$). Learning assessments that differ significantly are indicated by thin horizontal lines below the graph (for STIM session: yellow; for NOSTIM session: green; post hoc pairwise analysis, $p < 0.03$; $n = 8$). Since FDI was prioritized when identifying the TMS hotspot and rest motor threshold, both ADM and OP measurement taken at day 1 were highly variable across sessions even though subjects followed an identical protocol on that day. Nevertheless, for the ADM a similar decrease of the FacIndex from Mor D1 to Eve D1 was apparent which was only renormalized after unperturbed sleep (i.e. NOSTIM). The OP which was not activated by the tapping task exhibited a different pattern. For the NOSTIM session the FacIndex was close to 1 for all learning assessments, i.e. corticomotor excitability changed only minimally in response to motor training. After the STIM night, by contrast, the FacIndex was clearly < 1, indicating that corticomotor excitability was suppressed at the POST measurement compared to the PRE measurement (post hoc pairwise analysis, $p < 0.03$; $n = 8$). * indicates a significant stimulation session × learning assessment interaction ($p < 0.05$; $n = 8$); vertical bars indicate SEs. All post hoc tests were corrected for multiple comparisons in line with the modified Bonferroni procedure. See methods for further details.

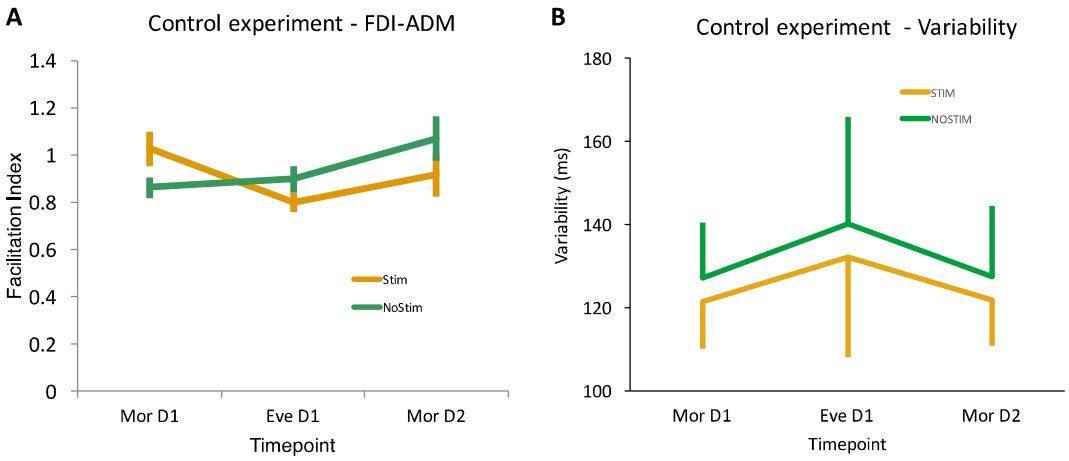

Figure S7.

Facilitation Index (A) and tapping Variability (B) are shown for both the STIM (yellow) and the NOSTIM session (green). Note that the Faciltation Index was highly variable at the Morning of D1, most likely because we had limited control about the subjects' behaviour at the day before the experiment started. Importnatly, the FacIndex of STIM and NOSTIM was very similar at the Evenning of D1 and also overnight imporvements occurred to a similar extent. Tapping Variability was slightly lower in the STIM than in the NOSTIM session, however, the pattern across the timepoints was highly consistent such that in both session variability was higher in the evening than in the morning measurements.

	NONSTIM	STIM	P-values
Nr of Stimulations	0.00 ± 0.00	3143.69±354.73	
NREMS [min]	318.8±5.7	320.4±5.0	0.68
N1 [min]	21.0±2.2	24.2±1.8	0.22
N2 [min]	167.3±9.9	178.4±9.8	0.10
N3 [min]	130.5±10.6	117.9±9.8	0.05
REMS [min]	99.6±3.6	92.9±3.7	0.12
TST [min]	418.4±5.6	413.3±5.6	0.28
SEF [%]	93.9±0.9	93.9±0.7	0.96
SL [min]	12.2±2.4	12.2±2.6	0.96
WASO [min]	18.6±3.6	17.8±1.9	0.77
Nr WASO	34.1±3.6	35.4±3.0	0.72
Nr Arousal	24.3±2.4	23.5±2.8	0.73

Table S1

Main experiment: Visually scored sleep variables for both sessions (mean ± SEM; paired t-test; n = 13 NREMS = NREM sleep; N1-N3 = NREMS stage 1-3; REMS = REM sleep; TST = total sleep time; SEF = sleep efficiency; SL = sleep latency; WASO = wake after sleep onset; Nr WASO = number of awakenings after sleep onset).

The difference between the two nights in N3, and N2 sleep did not correlate with the observed changes in the Facilitation index and variability during the finger tapping task (Spearman correlation p>0.35). These results reinforced our assumption that the observed difference in N3 sleep in the current study sample is rather a random effect and not due to the acoustic stimulation.

	Variability		Facilitation index	
Power	**RHO**	**p**	**RHO**	**p**
lowSWA Cluster	0.36	0.25	-0.37	0.34
Beta Cluster 1	0.11	0.75	-0.52	0.16
Beta Cluster 2	-0.01	0.99	-0.37	0.34

Table S2

Summary of the Spearman correlation coefficients (RHO and p values) between clusters (mean over all electrodes within the cluster), revealing any power differences between the two nights (considering all frequency band from Fig S3) and changes in Facilitation index or variability. For lowSWA one cluster located over the left sensory-motor cortex, and for beta power two clusters (cluster1 located over frontal cortex and cluster 2 located over right central and temporal cortices, for topographical details see Fig S3) were found.

		Mean_NoStim ± SEM	Mean_Stim ± SEM
Tiredness	Morning 1	4.43 ± 0.32	4.69 ± 0.71
	Evening	4.62 ± 0.48	5.12 ± 0.42
	Morning 2	5.68 ± 0.31	5.52 ± 0.47
Mood	Morning 1	6.54 ± 0.61	6.93 ± 0.73
	Evening	6.16 ± 0.47	6.26 ± 0.37
	Morning 2	5.87 ± 0.39	6.08 ± 0.31
Energy	Morning 1	4.38 ± 0.40	4.12 ± 0.82
	Evening	4.57 ± 0.46	5.51 ± 0.23
	Morning 2	5.15 ± 0.36	5.37 ± 0.30
Calm	Morning 1	7.17 ± 0.80	7.48 ± 0.66
	Evening	7.04 ± 0.33	6.90 ± 0.35
	Morning 2	6.73 ± 0.37	6.92 ± 0.32
Focus	Morning 1	5.41 ± 0.41	5.98 ± 0.60
	Evening	6.23 ± 0.41	5.62 ± 0.31
	Morning 2	5.45 ± 0.29	5.37 ± 0.33
Motivation	Morning 1	3.54 ± 0.31	3.63 ± 0.46
	Evening	3.26 ± 0.38	3.96 ± 0.45
	Morning 2	3.75 ± 0.37	4.22 ± 0.39

Table S3

Summary of the psychological questionnaires. Tiredness, Mood, Energy, Calm, Focus, Motivation: 0 = not tired, very bad mood, full of energy, very restless, not focused, very motivated; 10 = very tired, very good mood, no energy, very calm, very focused, not motivated. Note, mixed effect models revealed no significant difference between STIM and NOSTIM night ($p>0.12$).

	NOSTIM (mean±SEM)	STIM (mean±SEM)
Tiredness after sleep	4.51 ± 0.48	4.22 ± 0.37
Restfulness of sleep	4.53 ± 0.28	4.09 ± 0.23
Sleep depth *(compared to normal sleep)*	4.82 ± 0.31	4.50 ± 0.31
Sleep depth *(compared between nights)*	4.78 ± 0.30	4.22 ± 0.30

Table S4

Summary of the subjective quality of sleep based on questionnaires. Tiredness after sleep, Restfulness of sleep: 0 = very tired, not restful; 9 = not tired, very restful. Sleep depth (compared to normal sleep): 0 = lighter sleep than normally; 9 = deeper sleep than normally. Sleep depth (compared between nights): 0 = light sleep; 9 = deep sleep. Note, repeated measures ANOVAs showed no significant difference between STIM and NOSTIM night ($p>0.12$).

	Reaction time [ms]	
	Evening	Morning
STIM	254.24±31.05	249.22±21.83
NOSTIM	268.52±41.74	257.82±52

Table S5

Reaction time of the auditory attention task which was performed in the evening and morning to assess vigilance. Note, no significant difference between STIM and NOSTIM night was found ($p>0.12$).

To control for general changes in psychological conditions subjects filled in a psychological questionnaire (assessing attention, mood, concentration and motivation on a visual analog scale) prior to each learning session (Moring 1, Evening 1 and Morning 2). In addition, subjects were asked to rate their subjective sleep quality (based on an analog visual scale) in the morning after the EEG recordings. There were no changes in any assessed variable between the two sessions (see Table S5/S6), indicating that our behavioral and neural measurements were unlikely confounded by lack of attention, motivation or other side effects typically associated with general sleep deprivation.

Moreover, subjects completed an attention task (based on an auditory oddball paradigm) in the evening before and in the morning after sleep (~15min before bed time and 15 min after wake up) to control for vigilance state (i.e. sleep inertia). Reaction times, wrong clicks and missed clicks were assessed as performance score (for details see methods)

	n	Activity score/min day before the experiment	Activity score/min day day of the experiment
NOSTIM	13	393.16±28.15	370.06±24.41
STIM	12	386.37±23.99	354.60±33.01
p-Values		0.57	0.94

Table S6.

Summary of the activity counts /min of the day before the first experimental session and the day of the experimental sessions. Activity was recorded with a wrist actigraph (Actiwatch, subjects 1-7 and Geneactiv subjects 8-13, in one subjects the actigraph did not work prior to the STIM session). Activity counts/min were calculated for Actiwatch recorders according to the Actiwatch software (Actiwatch. Actiware - Tutorials: http://www.actigraphy.com/devices/actiware/tutorials.html), and for Geneactiv recorders according to the algorithm described by Van Someren and te Lindert (2013).

	NONSTIM	STIM	P-values
Nr of Stimulations	00±00	2663±541	
NREMS [min]	311.76±4.6	321.38±6.9	0.08
N1 [min]	24.95±2.62	21.62±5.0	0.46
N2 [min]	191.71±5.82	209.62±5.0	0.02
N3 [min]	95.10±8.25	90.38±10.48	0.53
REMS [min]	111.57±5.0	109.33±6.28	0.80
TST [min]	423.33±8.55	430.71±5.61	0.33
SEF [%]	93.24±1.24	95.14±0.66	0.21
SL [min]	14.43±3.64	10.05±1.88	0.13
WASO [min]	19.95±3.46	14.48±3.23	0.30
Nr WASO	36.14±6.85	34.14±7.92	0.21

Table S7

Control experiment: Visually scored sleep variables for both sessions (mean ± SEM; paired t-test; n = 7; NREMS = NREM sleep; N1-N3 = NREMS stage 1-3; REMS = REM sleep; TST = total sleep time; SEF = sleep efficiency; SL = sleep latency; WASO = wake after sleep onset; Nr WASO = number of awakenings after sleep onset).

5. Discussion

The overall goal of this work was to investigate the role of sleep slow waves in the regulation of synaptic homeostasis in humans. We did so by following 3 different research aims. First, we investigated different electrophysiological markers of network connectivity and sleep homeostasis in the human wake and sleep EEG during normal development. We could show that during the first year of life, the slope of slow waves decreases during sleep, presumably reflecting the dissipation of sleep pressure. Furthermore, at late childhood when synaptic density is highest, the drive of sleep pressure during the day might be very strong. We found that at that age theta waves in the wake EEG might reflect local sleep. In addition, ApEn a marker of long-distance connectivity significantly increased with age, presumably reflecting the age-dependent improvement of the functional integration of distant brain networks. Second, we translated these findings to clinical population with disturbed slow wave sleep to focus on the function of slow waves on synaptic homeostasis. To do so, we analysed the overnight dynamic of the slope of slow waves in children diagnosed with a childhood epilepsy, characterized by an activation of spike wave activity during slow wave sleep (*i.e.* CSWS and West syndrome). We did not only find that the physiological overnight decrease of the slope of slow waves was diminished in these patients, but also found some evidence that this observation was related to the epileptiform abnormalities in the EEG. Third, we applied a novel tool to assess causality between sleep slow waves and the restorative function of sleep on synaptic strength. Therefore we develop a closed-loop auditory stimulation tool to locally perturb slow waves over the primary motor cortex and assessed the consequences on behavioural and neurophysiological markers of neuroplasticity arising from dedicated motor practice. We showed that the capacity to undergo neuroplastic changes is reduced by wakefulness but restored during unperturbed sleep. However, this restorative process was diminished when slow waves were locally perturbed in the primary motor cortex. This demonstrates that deep sleep is a requirement for maintaining learning efficiency.

5.1 Different indirect electrophysiological markers of network connectivity and sleep homeostasis during healthy human development

In the mature adult brain, synaptic strength is tightly regulated: It increases during wakefulness and decreases during sleep (Liu et al., 2010; Vyazovskiy et al., 2008). However, despite massive ongoing synaptic turnover (Zuo et al., 2005), the total number of synapses remains stable (Huttenlocher, 1979b). This means that diurnal changes in synaptic strength are mainly related to synaptic potentiation or depression (*e.g.* phosphorylation/dephosphorylation of AMPA receptors and CaMKII, or AMPA receptor turnover, Vyazovskiy et al. 2008). Since these changes in synaptic strength are related to brain states (*i.e.* increase during wakefulness and decrease during sleep), they have been linked to the regulation of the wake-sleep cycle (Tononi and Cirelli, 2014, 2006, 2003). In contrast, brain development represents a time of profound synaptic remodelling with vast synaptogenesis during early infancy and childhood, followed by massive synaptic pruning throughout adolescence (Huttenlocher, 1978; Zuo et al., 2005). Compared to the daily synaptic changes across the sleep-wake cycle, these morphological changes in synaptic strength (*i.e.* formation and elimination of synapses) occur over a much longer time scale. Nevertheless, they have also been linked to the process of sleep homeostasis. Ringli and Huber

(Ringli and Huber, 2011) proposed an imbalance of the wake and sleep-dependent regulation of synaptic strength during development: in the early years, synaptic strengthening during wakefulness (*i.e.* potentiation and formation) prevails over synaptic weakening (*i.e.* depression and elimination) during sleep, resulting in progressive build-up of synapses. Contrarily, during adolescence, the scale tips to the other side: synaptic downscaling during sleep overcomes synaptic strengthening during wakefulness leading to an overall decrease in synapses. Once the brain is mature, the scale is balanced across the wake-sleep cycle, attaining a stable level of synaptic strength. This notion is supported by the experimental findings that in young mice the number of spines and filopodia was increased during the dark period (when mica are mostly awake) and decreased after the light period (when mice are mostly asleep, Yang & Gan 2012). In contrast, in adult mice, spine turnover was stable across the sleep-wake cycle (Maret et al., 2011). Accordingly, Olini and Huber (2014) observed in a longitudinal study an overall gain of SWA across 24h in young rats, which was followed by a net loss of SWA with increasing age and was balanced across 24h when the animal reached adulthood.

5.1.1 Indirect markers to assess the development of sleep homeostasis and structural changes in cortical connectivity in the wake and sleep EEG

During the first year of life, SWA does not follow a continuous decline during sleep (Jenni et al., 2004). One could speculate that compared to the profound ongoing synaptic formation, the sleep and wake-dependent modulation of synaptic strength might be relatively small. Hence, a more sensitive marker for the level of synchronized neuronal network activity (*i.e.* the slope of slow waves) might be needed to depict such minor changes in synaptic strength. In line with this assumption we found on overnight reduction of the slope of slow waves in infants at the age of 2 to 9 months. Moreover, the slope rose with age, which was topographically most pronounced over the occipital cortex (research article 1). This finding resembles the developmental trajectory pattern of peak synaptic density (Huttenlocher and Dabholkar, 1997). Thus, our results provide indirect evidence that the slope of slow waves in the human EEG might reflect changes in synaptic strength. On the one hand, higher synaptic strength (*i.e*. by increased synaptic density) in the occipital cortex compared to more frontal areas could result in improved synchronized transitions between on-and off-states of the slow oscillation within neuronal population. This is reflected in steeper slow waves over the occipital cortex. On the other hand, synaptic downscaling (*i.e*. depression or elimination) during sleep might reduce the level of neuronal network synchronization. This is reflected in an overnight decrease of the slope of slow waves.

In contrast to the first year of human brain development, which represents the early stage of synaptic formation, at late childhood, synaptic density peaks at its maximum (Huttenlocher, 1978). Thus, at the time when the overall level of synaptic strength is highest, the capacity to further increase synaptic strength during wakefulness might be close to its limit. Consequently, only little additional wake-dependent potentiation of synaptic strength might be sufficient to induce high level of sleep pressure (Carskadon et al., 2004; Jenni et al., 2005) . Such accelerated build-up of sleep pressure might lead to an intrusion of sleep-like brain activity in the evening wake EEG. Accordingly, in the second research article we found that theta waves in the wake EEG at that age resemble local off-states which were described on the cellular level in awake freely moving rats (Vyazovskiy et al., 2011). According to the

hypothesis of Vyazovskiy and Harris (2013), such local off-states during wakefulness may reflect precaution of the organism in order to prevent irreversible cellular damage. It is assumed that sustained neuronal activity during waking may result in multiple forms of cellular stress. These lead to an accumulation of cellular damage. The off-states during subsequent slow wave sleep are thought to prevent the neurons with a time period to perform cellular maintenance and restore cellular homeostasis. Thus, local off-states during waking may serve as a similar opportunity to prevent the accumulation of further cellular wear and tear. However, so far no evidence for such a recuperative function of local off-states during wakefulness exists. One possibility to further investigate this hypothesis would be to disturb or prevent the occurrence of such local off-states. If such local off-states are similar to the global off-states underlying slow wave sleep, one could try to manipulate them by sensory stimulation. For instance, in an animal model one could deliver tones time-locked to the occurrence of local off-states and asses the resulting consequences on neuronal homeostasis (*i.e*. by quantifying markers of cellular stress). Nevertheless, despite a possible recuperative function of local off-states during waking, the occurrence of such local off-states may also have some negative consequences in terms of behaviour and performance. Reliable sensory information processing could be impaired in neurons which undergo off-states during wakefulness (Vyazovskiy et al., 2011, 2012). In fact, theta activity increase in the human wake EEG has been related to performance decrease after sleep deprivation (Cajochen et al., 1999; Makeig et al., 2000). Thus, local populations of cortical neurons falling asleep could be responsible for the impaired performance after sleep deprivation.

Due to the retrospective design of our study, we were not able to control for circadian influences. This certainly limits the interpretation of our results. As highlighted in the introduction, process C counteracts the increase of sleep pressure in the evening to sustain stable performance level (Cajochen et al., 2002). Hence, future studies based on sleep deprivation protocols are needed, to further investigate whether theta waves in the surface wake EEG mirror local sleep on the neuronal level.

5.1.2 Structural and functional brain development - do slow waves play a role?

As outlined above, slow waves are thought to mirror synaptic strength (Tononi and Cirelli, 2014). This assumption concurs with our findings that characteristics of slow waves in the EEG (in particularly the slope of slow waves) might map changes in synaptic strength while asleep (*i.e.* sleep homeostasis), as well as, during development when massive formation of new synapses occur. The vast build-up of synapses during infancy and childhood is followed by synaptic elimination during adolescence (Huttenlocher et al., 1982; Zuo et al., 2005). This maturational pruning of synapses occurs asynchronously in different brain areas and can also be mapped by SWA (Kurth et al., 2010b). Topographical predominance of SWA across development follows a posterior to anterior time course, which parallels the regional trajectory of peak cortical thickness (Buchmann et al., 2010; Kurth et al., 2010b; Shaw et al., 2008). These morphological changes of cortical development are accompanied by a refinement of neuronal circuits to establish a more meaningful and specific cortico-cortical connectivity (Lichtman and Colman, 2000). Thus, the temporal pattern of morphological brain maturation is paralleled by the development of cognitive functions (Gogtay et al., 2004; Paus, 2005). For example, while functions relying on the occipital cortex (*e.g.* visual skills) reach adult level already within the first years

of life (Teller, 1981), other functions depending mainly on the frontal cortex (*i.e.* cognitive functions such as working memory) progressively mature from childhood to adulthood (Luna and Sweeney, 2004). The development of such higher order cognitive functions is thought to rely not only on pruning processes but also on increasing myelination of fibre tracks (Luna and Sweeney, 2004), which continuous into young adulthood (Paus et al., 2001). Such progressive growth of the myelin sheath might increase efficient information transfer to improve the functional integration of distant brain regions. In fact, a functional MRI study including participants aged 7 to 31, revealed a strong tendency towards increased long-range connections with age (Fair et al., 2007). Further support for this notion comes from our third study (research article 3), in which we investigated age-dependent differences in ApEn, a measure which is thought to reflect differences in long-range connectivity. Higher ApEn values are associated with better long-range connectivity (Pincus, 1994). In our analysis, adults showed significantly higher ApEn values compared to teenaged children over the central and frontal cortex, brain areas which are not fully developed at this age. However, ApEN values over more posterior regions did not differ between children and adults. These findings are in line with structural data, showing that maturation of the occipital cortex reaches adult level shortly before puberty (Huttenlocher and Dabholkar, 1997). Thus, an increase in long-range connectivity with age might improve the integration of different brain networks, which may further contribute to the age-related improvement of demanding cognitive tasks (Fair et al., 2008).

In sum, the functional maturation of the human brain seems to be linked to the morphological development of brain structures. We have highlighted these structural changes during development (*i.e*. focusing on synaptic strength) and how they might be regulated in correspondence with the wake-sleep cycle. We have presented different indirect markers in the human surface EEG, which might allow to map this regulatory processes. Notably, slow waves during NREMS, seem to present a suitable marker to follow the regulation of synaptic strength, not only across the night, but also in the course of development. Thus, as proposed by Ringli and Huber, the wake and sleep-dependent modulation of synaptic strength day after day, might slowly shape cortical maturation from early infancy to adulthood (Ringli and Huber, 2011). However, this raises the question about the role of slow waves: Are slow waves merely mirroring changes in synaptic strength or are slow waves playing a causal function? It has been proposed that the neuronal activity pattern underlying slow waves, the slow oscillation, might actively contribute to synaptic downscaling during sleep (Tononi and Cirelli, 2014). The slow oscillation, consisting of synchronized bursts of firing and neuronal silence, might be ideally suited to induce synaptic depression (Czarnecki et al., 2007). Thus, in a recent study combining MRI and sleep EEG measures, Kurth et al. (2012) reported that SWA was predictive of grey matter and skill maturation, providing first evidence for an active role of slow waves during human development. However, for the ultimate proof of causality, a direct manipulation of either slow waves or synaptic strength would be needed.

In a first intermediate step, a simpler alternative is to investigate markers of sleep regulation (*i.e*. wake and sleep-dependent changes of synaptic strength) in children with disturbed slow wave sleep. Thus, in the second part of this thesis project, we analysed the overnight changes of the slope of slow waves in

children diagnose with an epilepsy characterized by an activation of epileptiform discharges during slow waves seep.

5.2 Evidence for an impaired sleep-dependent decrease of synaptic strength in patients suffering from CSWS or West syndrome

Slow wave sleep is strongly altered in children diagnosed with an epilepsy characterized by an activation of epileptiform discharges (*i.e.* spike wave activity) during slow wave sleep, such as CSWS or West syndrome (for a representative example see introduction, Figure 3). Together with a disturbed slow wave sleep, the mental development is often deteriorated in these patients. This is why the pathological epileptiform abnormalities are thought to damage normal brain functioning (ILAE, 1989). On the assumption that slow waves are required for the sleep-dependent renormalization of synaptic strength (Tononi and Cirelli, 2014), one might pose the question whether synaptic downscaling is disturbed in these children. As stated above, synaptic downscaling during sleep may be important for the tight regulation of the wake and sleep-dependent changes of synaptic strength across 24 hours. Over the long term, such homeostatic regulation of synaptic strength is assumed to account for physiological brain development (Ringli and Huber, 2011). Moreover, there is experimental evidence that not only in the long run but also on a daily time scale, sleep slow waves are important for learning processes. For example, enhanced SWA, either by a use-dependent increase of synaptic potentiation, or by electrical current stimulation were associated with a sleep-dependent performance improvement (Huber et al., 2004; Marshall et al., 2006). Thus, in CSWS and West syndrome patients, spike wave activity during slow wave sleep may impair physiological synaptic downscaling, which could account for the observed developmental retardation. In line with this hypothesis, Bölsterli et al. (2011) could show that the slope of slow waves did not decrease during sleep in CSWS patients.

5.2.1 Spike wave activity is related to impaired renormalization of the slope of slow waves during sleep in CSWS patients

Capitalizing on the work of Bölsterli et al. (2011), a follow-up study was conducted to further elaborate the relationship between the impaired overnight reduction of the slope of slow waves and spike wave activity (research article 4). Assuming that epileptiform discharges during slow wave sleep may disturb the physiological renormalization of synaptic strength, spike wave activity and impaired slope reduction should be related. Indeed, we found that the reduction of the slope of slow waves was most diminished in the epileptic focus. In addition, the density of spike waves was negatively correlated with the overnight slope decrease. Thus, the more spike waves were observed, the less the slope decreased. These results provide further evidence for a spike dependent pathophysiological mechanism during sleep for the disturbed renormalization of synaptic strength. A recent study with patients suffering from pharmaco-resistent focal epilepsy who underwent combined scalp and intracerebral EEG recordings for diagnostic purposes shed further light on the relationship between spike wave activity and sleep slow waves (Frauscher et al., 2015). The authors found that epileptic activity during slow wave sleep is mainly mediated by high amplitude slow waves. Also, in contrast to physiological activity which occurs during the on-state, epileptic activity arises at the transition from the on- to the off-state, the time of maximized synchronicity (Volgushev, 2006). This results suggest that periods of high synchronization might

facilitate the occurrence of epileptic activity (Frauscher et al., 2015). In the course of development, CSWS emerge during childhood, the time when synaptic density and thus, sleep slow wave amplitude, are highest (Feinberg, 1982). Furthermore, the bistability of cortical neurons during NREMS represents a condition when neuronal network supports both the distribution and synchronization of activity among the network (Massimini et al., 2004; Sanchez-Vives and McCormick, 2000; Timofeev et al., 2000). Hence, high synaptic strength at that age and presumably other mechanisms (*e.g.* altered intrinsic currents due to extracellular increased K+ or decreased Ca2+ concentrations, Timofeev et al., 2012), may increase neuronal synchronization during NREMS above a certain threshold: Physiological slow waves, then, become spike waves (Steriade and Amzica, 1994). Thus, through the highly connected network, the entire cortex starts spiking during NREMS, which corresponds to the pathophysiological picture of sleep in CSWS patients. Such spiking activity could interfere with the physiological activity pattern underlying sleep slow waves and therefore prevent synaptic downscaling.

In conclusion, spike waves during sleep might impair the slow wave dependent renormalization of synaptic strength. Over time, such altered long-lasting synaptic homeostasis might be the basis for the development of neurophysiological deficits described in CSWS patients. In line with this assumption, Urbain at al. (2011) reported a renormalization of sleep-dependent memory consolidation after successful treatment of spike wave activity in CSWS patients. However, due to the retrospective study design of our study, we were not able to directly link spike wave activity and altered overnight slope decrease to neurobehavioral outcomes. Thus, future studies are needed, which combine the assessment of spike wave activity, the overnight slope decrease and neurocognitive testing.

5.2.2 Sleep-dependent decrease of the slope of slow waves might be altered in West syndrome patients due to hypsarrhythmia

In a second follow-up study (research article 5), we investigated the overnight reduction of the slope of slow waves in infants diagnosed with West syndrome. Compared to the continuous spike wave activity in CSWS during NREMS, the EEG of West syndrome patient is characterized by hypsarrhythmia (*i.e*. random, high voltage spikes and slow waves with variable amplitudes). Similar to the CSWS patients, the overnight reduction of the slope of slow waves was impaired in West syndrome patients, which was related to the pathological EEG pattern of hypsarrhythmia. Thus, in line with the above presented results of the CSWS study, the pathological epileptiform discharges during slow wave sleep may be responsible for an altered slow wave dependent regulation of synaptic strength. Compared to CSWS which occurs during childhood, West syndrome occurs in the first year of live (Hancock et al., 2009). During this stage of brain development, a tremendous quantity of new synapses is formed to develop a highly connected cortico-cortical network (Huttenlocher, 1990). Such an increase of network connectivity may facilitate the development of globally synchronized slow oscillations during slow wave sleep. In line with this suggestion, we could show that the amount of globally synchronized slow waves increased with age during the first year of live in healthy infants (Fattinger et al., 2014, first research article). In contrast, hypsarrhythmia is characterized by chaotic EEG activity and thus, never reflects as a highly organized EEG pattern (Watanabe et al., 1993). Hence, in a preliminary analysis, we assessed the proportion of globally synchronized slow waves in West syndrome patients. Before treatment, the proportion of global

slow waves was reduced in West syndrome patients compared to healthy controls (patients: 12.8 ± 1.6%; controls: 23.2 ± 1.9%; p<0.0001; unpublished data). However, after treatment (*i.e.*, no hypsarrhythmia), patients showed a similar proportion of global slow waves compared to healthy controls (p=0.92; unpublished data). In a recent review, Vyazovskiy and Harris hypothesized that a steady slow oscillation within neuronal networks requires spatially highly synchronized activity. Owing to the extensive recurrent circuitry of the cerebral cortex, the susceptible neuronal off-periods of the slow oscillation need to be globally coordinated (Vyazovskiy and Harris, 2013). Thus, ongoing asynchronous neuronal activity due to hypsarrhythmia may easily interrupt and dissolve off-states of single neurons, and thereby interferes with the slow oscillation. To sum up, asynchronous, chaotic network activity due to hypsarrhythmia might impair the generation of the physiological slow oscillation during NREM sleep and thereby impairs synaptic downscaling in West syndrome patients.

In conclusion, in both West syndrome and CSWS patients, slow wave sleep might be disturbed due to pathological epileptiform discharges. Both childhood epilepsies occur during specific time windows of development. We proposed two different pathophysiological mechanisms for the two epilepsies which were linked to these specific developmental stages of cortical maturation: West syndrome emerges during the first year of live, a period of early synaptic formation and white matter development (Huttenlocher, 1990; Paus et al., 2001). A global synchronization of the slow oscillation may, thus, not yet be fully established. Therefore, during this time window, chaotic epileptic activity (*i.e.* hypsarrhythmia) might further impair the generation of spatially synchronized neuronal activity which is necessary to maintain a stable slow oscillation during NREMS. On the other hand, CSWS occurs during childhood, the time when synaptic density is highest. At that age, the degree of synchronized neuronal activity during NREMS may increase above a certain threshold when physiological slow waves become spike waves. This hypothesis indicates that physiological slow wave sleep may require a tightly regulated balance of synchronized network activity.

Taken together, the results of the second part of this thesis, *i.e.* the retrospective analysis of the slow wave dependent regulation of sleep homeostasis in children with disturbed slow wave sleep, provide some further evidence for an active role of sleep slow waves for the regulation of synaptic homeostasis in humans. Nevertheless, these results remain correlative. Moreover, the analysis of the sleep EEG of CSWS and West syndrome patients requires an intensive pre-processing of the data due to artefacts related to the epileptiform discharges, which certainly limits the interpretation of the results.

5.3 Selective manipulation of sleep slow waves in humans

There is direct evidence on the molecular (Vyazovskiy et al., 2008), electrophysiological (Liu et al., 2010), and the structural (Bushey et al., 2011) level for a sleep and wake dependent regulation of synaptic strength. SHY claims that sleep slow waves, on the one hand mirror synaptic strength and on the other hand, actively decrease synaptic strength during sleep (Tononi and Cirelli, 2014). However, the former studies mentioned above rely all on data obtained from cortical slices of animals which had to be sacrificed (Bushey et al., 2011; Liu et al., 2010; Vyazovskiy et al., 2008). Therefore, up to date, a direct proof that the sleep-dependent changes in synaptic strength are linked to sleep slow waves does

not exist. Nevertheless, there are indirect methods, which allow to assess changes in synaptic strength *in vivo*. For example, synaptic efficacy can be tracked by the slope of the LFPs evoked by electrical stimulation in animals (Vyazovskiy et al., 2008). Similarly, in humans, changes in cortical excitability can be assessed by means of TMS (Esser et al., 2006). Both methods have been used to demonstrate that the sleep-dependent reduction of synaptic strength is related to sleep slow waves (Huber et al., 2013; Vyazovskiy et al., 2008). Though, to further proof that sleep slow waves are causally involved in synaptic downscaling during sleep, one needs to selectively manipulate sleep slow waves and directly assess the consequences.

SHY claims that the neuronal firing pattern underlying slow wave sleep, the slow oscillation between on- and off- states, drives synaptic downscaling during sleep (Czarnecki et al., 2007; Lubenov and Siapas, 2008). A more recent hypothesis (*i.e*. sleep for the single neuron) claims that not only the firing pattern alone but also the off-states per see are important to restore synaptic and neuronal homeostasis during sleep (Vyazovskiy and Harris, 2013). However, these off-states are not stable and might be easily interrupted by synaptic input from other neurons. Thus the neuronal off-state might present a vulnerable time window to manipulate the ongoing slow oscillation during slow wave sleep. Accordingly, a recent study reported that SWA was decreased compared to a normal night, if auditory stimulation were delivered time-locked to the down-phase of slow waves during sleep (Ngo et al., 2013). Based on this background, and the knowledge that slow waves are locally regulated in humans (*e.g* Huber et al., 2004, for a more detailed discussion see below), we aimed to focally disturb the ongoing slow oscillation during sleep. Therefore, we developed a closed-loop auditory stimulation tool to detect slow waves in real time.

5.3.1 The closed-loop auditory stimulation tool: local perturbation of slow waves

Already in the 1930s when pioneering sleep research discovered the different sleep stages in the human surface EEG, it was recognized that slow waves occur synchronously over the entire cortex (Berger, 1929; Blake et al., 1939). This observation led to the prevailing view that slow wave sleep represents a global brain phenomenon. However, thanks to the technical progress over the past few decades (*i.e*. hd-EEG, Positron emission tomography (PET), functional MRI), further research has demonstrated that individual slow waves do not involve the entire cortex homogeneously (Dang-Vu et al., 2008; Huber et al., 2004; Maquet, 2000). For example, hd-EEG studies found a use-dependent regulation of slow waves, probably related to LTP/LTD dependent changes of synaptic strength (Huber et al., 2006, 2004). Further investigation of single slow waves, including source modelling, revealed that each individual wave is an idiosyncratic event, propagating across the cortex from distant origins (Massimini et al., 2004; Murphy et al., 2009). More direct evidence for local slow waves in humans comes from neurosurgical patients implanted with multi-region intracerebral depth EEG electrodes (Nir et al., 2011). This study has shown that even though some slow waves occur concurrently in a number of distant brain regions, most slow waves are confined to local brain areas (Nir et al., 2011). Thus, by combining our closed-loop auditory stimulation tool with hd-EEG recording, we intended to examine, whether slow waves can be disturbed locally. Therefore, slow waves over a certain brain area (*i.e*. by selecting one electrode of the hd-EEG net) were detected in real time, and auditory stimuli were played time-locked to the down-phase of the slow wave. Thereby, we aimed to hit the vulnerable off-state of the underlying cortical neurons.

Since slow waves are locally regulated, the tones are only systematically time-locked to the down-phase of the slow waves in the selected brain area, whereas in the remaining brain, tones are randomly presented at any phase of the ongoing slow wave cycle. With this approach, SWA should be focally reduced over the selected brain area, without changing the global structure of sleep (*i.e*. global amount of SWA or sleep architecture).

As demonstrated in our research article 6 we were able to locally reduce SWA over primary motor cortex without changing the global structure of sleep. Such local slow wave manipulation due to auditory stimulation was further confirmed by different pilot recordings. As shown in Figure 4 SWA can be locally reduced over different brain areas. Two examples of single subjects (left frontal and left motor-sensory SWA reduction, Figure 4A) and one group example including 11 subjects (right parietal SWA reduction, Figure 4B) are shown (unpublished data).

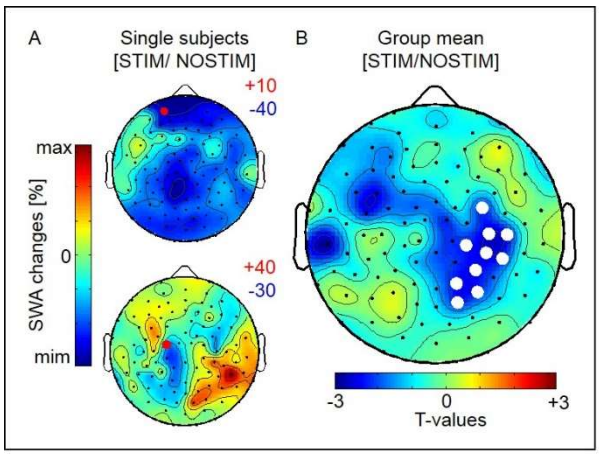

Figure 4

Local reduction of SWA (1-2 Hz) over different brain areas. (A) Topographical SWA reduction [%] of two single subjects are shown: In the upper example, auditory stimulation was time-locked to the down-phase of detected slow waves over left frontal and in the bottom example over left somatosensory cortex (indicated by a red dot, numbers in the upper right corner indicate maximal and minimal changes in SWA [%]). Note, auditory stimulation induced a focal reduction of SWA over the respective brain area. (B) Statistical comparison of topographical reduction of SWA over the right parietal cortex in a group of 11 healthy subjects (mean age 23 years, all men). Auditory stimulation was time-locked to the down-phase of detected slow waves over right parietal cortex. Blue colours indicate a decrease and red colours an increase in SWA in the STIM compared to the NOSTIM condition. Note during STIM a significant reduction of SWA by ~12±4% was found in a local cluster of 10 electrodes (white dots, $p < 0.05$, paired t-test, after nonparametric cluster-based statistical testing; STIM = stimulation night; NOSTIM = non-stimulation night; data not published).

As described in research article 6, the accuracy of our closed-loop algorithm to detect slow waves in real time was verified. Compared to other groups, our approach to perform online slow wave detection is rather simple. It depends on an amplitude threshold criteria and does not take into account any information about phase-timing. More specifically, after re-referencing and filtering (between 0.5-2 Hz), the EEG signal of the stimulation electrode (*i.e*. located over the brain region where we want to focally disturb slow waves) is monitored. Every time the EEG signal crosses a certain negative threshold (*e.g.*

-30µV), a tone (pink 1/*f* noise of ~50dB, 50ms duration) is delivered to the subject. The advantage of this simple approach is that the detection of slow waves depends on the instantaneous signal (*i.e*. it does not depend on any predictions). However, the flip side of the coin is that no information about the phase of the ongoing slow wave is considered. Moreover, the filtering of the EEG signal induces a phase shift, which changes with the frequency. Thus, although the majority of the tones were presented during the down-phase of the slow waves (*i.e*. between the negative and the positive zero crossing of the signal), it was not very accurate in delivering the tones at a certain phase of the slow wave cycle. A detailed offline, post-hoc phase-timing analysis revealed that tone onset was distributed over the entire down-phase of the slow wave (Figure 5A). This variability of tone onset was in line with the observed variability of local SWA reduction across subjects. For a more detailed analysis of the phase timing the negative half wave was divided into 30°- phase bins and the percentage of tones presented during each 30°- phase bin were quantified (Figure 5). We found that the local SWA decrease was most successful, if most of the tones were applied after the negative peak of the slow wave cycle during section 5 (Figure 5 B, Spearman's RHO coefficient -0.74, $p<0.01$; unpublished data).

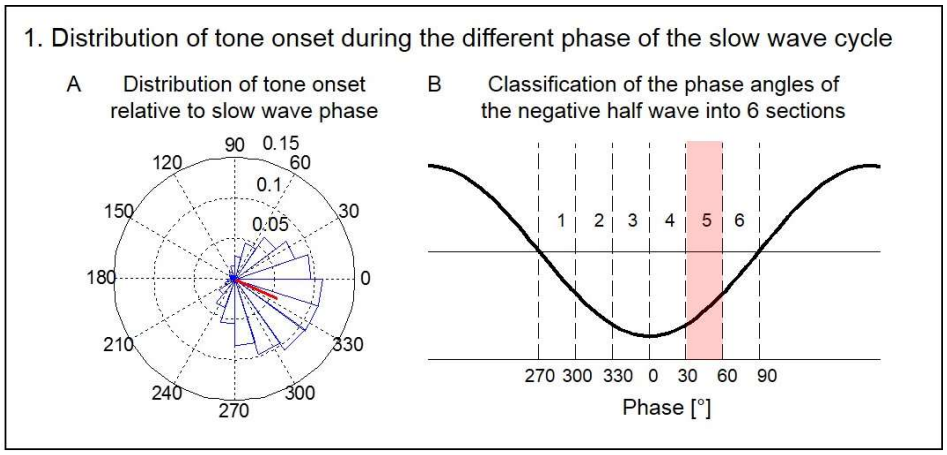

Figure 5

Offline verification of the closed-loop detection algorithm. (A) The distribution of tone onset relative to the phase of the slow wave cycle from all subjects (n=13). The red line represents the mean phase vector. Note, the majority of the tones (~ 80%) were delivered during the negative half wave (*i.e.* 270° - 360°/ 0° - 90°, analysis was performed with the CircStat MatLab toolbox; Berens, 2009). (B) Schematic illustration of the different phase angles of the negative phase of the slow wave. The negative half wave was divided into 6 30°-phase bins (section 1-6) for a more detailed analysis of the phase timing. Local SWA reduction was most prominent if tones were applied during section 5 (unpublished results, analysis was based on the data presented in research article 6).

In sum, online detection algorithms which deliver tones more precisely time-locked to a certain phase of the slow wave cycle might significantly increase the induced effect of auditory stimulation on slow waves. Different algorithms which include the phase of the ongoing oscillation for slow wave detection have been presented (Cox et al., 2014; Ngo et al., 2013; Santostasi et al., 2016). The algorithms suggested by Cox et al. (2014) and Ngo et al. (2013) depend on phase prediction, which is related to the history of the EEG signal. In contrast, the recently introduced phase-locked loop (PLL) by Santostasi and

colleagues (2016) represent a more sophisticated approach for online phase tracking of the EEG. Such a PLL consists of an adaptive feedback algorithm, which tracks the ongoing EEG signal and determines the underlying phase of the signal in real-time. Thus, by implementing a PLL into the auditory stimulation algorithm, tones can be delivered much more accurately and precisely time locked to a certain phase of the slow wave cycle (Santostasi et al., 2016). However, so far this stimulation approach has only been tested to enhance SWA, this is by presenting tones time-locked to the up-phase of the slow wave cycle. Whether such PLL also helps to improve SWA reduction needs to be elaborated in further studies.

Yet, to the best of our knowledge, we were the first group to investigate the local aspects of slow wave manipulation. Using hd-EEG, we could show that SWA can be locally reduced by auditory stimulation. A recent study compared the spatial and temporal resolution of K-complexes during slow wave sleep, evoked by different kinds of sensory stimulation modalities (Riedner et al., 2011). Overall, the authors found a consistent topographical distribution of the negative peak of the evoked K-complex (*i.e.* N550) for somatosensory, auditory and visual stimulation. This observation coincides with the view that peripherally induced slow waves underlie a common mechanism that does not directly depend on primary sensory pathways (Bastien et al., 2002; Colrain, 2005). However, source modelling of hd-EEG recordings revealed that the early components of the K-complex (*i.e*. P200 and N550) induced by visual and somatosensory stimulation, showed increased involvement of somatosensory and visual primary cortical areas. This is consistent with the activation of primary sensory pathways. However, for auditory stimulation, relatively few areas showed different involvement of the evoked K-complex (Riedner et al., 2011). These observations might explain why we were able to reduce SWA locally over different brain areas, using auditory stimulation. Nevertheless, manipulation of SWA over somatosensory and visual cortices might be even more focal, if one applies stimuli, which would be specific to the sensory modality. Thus, the reduction in SWA over left motor-sensory cortex as described in research article 6 could have been more locally restricted if somatosensory stimuli would have been applied.

Taken together, we have demonstrated that SWA can be locally reduced if auditory stimuli are presented time-locked to the down-phase of ongoing slow waves. In the following section the electrophysiological mechanisms which could underlay such slow wave perturbation will be discussed.

5.3.2 The off-state of cortical neurons as a time window to interfere with the ongoing slow oscillation

Slow waves during NREMS in the EEG reflect the underlying ongoing slow oscillation of cortical neurons; the up-phase of slow waves corresponds to the active on-state and the down-phase to the silent off-state (Vyazovskiy et al., 2009). Three primary active intrinsic currents of cortical neurons are involved in the generation of this slow oscillation. During the active on-state, the I_{DK} facilitates the termination of the on-state whereas during the off-state, the $I_{Na(p)}$ and I_h enable the initiation of the next on-state (Hill and Tononi, 2005). The hyperpolarized off-state has been related to a disfacilitation within the cortical network (Contreras et al., 1996). It has been proposed that phasic depletion of extracellular Ca^{2+} concentration during the active on-state may induce functional disconnection of cortical synapses (Massimini and Amzica, 2001). Sustained neuronal spiking activity of virtually all cortical neurons during

the on-state may result in a progressive decrease of extracellular Ca^{2+} level. This strongly reduces the probability of neurotransmitter release in the time course of the on-state. During the subsequent silent off-state, extracellular Ca^{2+} concentration, and thus, synaptic efficacy, is restored, resulting in a maximal likelihood of neurotransmitter release at the beginning of the following on-state (Massimini and Amzica, 2001). Moreover, low concentration of extracellular Ca^{2+} promotes electrical coupling of neurons and glia cells (*i.e.* by an increased effectiveness of gap junctions throng opening of hemichannels; Thimm et al., 2005). This facilitates the synchronization of inhibitory fast-spiking interneurons (Gibson et al., 1999). Such network mechanisms may eventually be strong enough to silence the entire network synchronously (Volgushev, 2006). On the other hand, the $I_{Na(p)}$ seems to be the most important current to initiate the on-state (Hill and Tononi, 2005). Several mechanisms have been proposed to depolarize a neuron sufficiently during the off-state to activate $I_{Na(p)}$ and, thus, to induce the next on-state. For example, it has been show that occasional summation of action potential-independent release of neurotransmitters (*i.e.* miniature EPSP, mEPSP) could activate $I_{Na(p)}$ and depolarize the membrane of cortical neurons sufficiently for spiking generation (Bazhenov et al., 2002; Chauvette et al., 2010; Timofeev et al., 2000). Moreover, the intrinsic I_h which is activated during the hyperpolarized off-state might also increase the membrane potential (Hill and Tononi, 2005). However, under normal physiological conditions, it is relatively weak and, thus, unlikely to play a significant role in promoting an on-state (Timofeev et al., 2012). Together, these findings are in line with the observation of the slow oscillation in athalamic animals (Steriade et al., 1993c), implying a cortical origin of the slow oscillation. However, despite the fact that the cortex might be sufficient to generate a slow oscillation (Steriade et al., 1993c), there is increasing evidence that a functional interaction of the thalamocortical network may be required for the full EEG manifestation of slow wave sleep (for review see Crunelli and Hughes, 2010). It has been demonstrated that cortical on-states can be elicit by thalamocortical input as effective and frequent as by intercortical input (Crunelli and Hughes, 2010). Furthermore, according to a computer model of the thalamocortical system, excitatory synaptic input (*i.e.* EPSP) from the thalamus might be capable of inducing an abrupt transition form off- to on-states (Hill and Tononi, 2005). In compliance with this assumption, simulation of sensory thalamocortical inputs lead to shorter off-states in pyramidal neurons, located postsynaptically to the thalamocortical neurons (Bazhenov et al., 2002). In addition, different intracellular studies revealed that compared to the on-state, EPSPs are more reliably elicited during the off-state. Also, their amplitude and duration is increased (Crochet et al., 2005; Léger et al., 2005; Petersen et al., 2003; Sachdev et al., 2004). Such an increase of EPSP amplitude was related to the extracellular Ca^{2+} concentration (Crochet et al., 2005). These latter authors found a progressive increase of the amplitude during the course of the off-state which was paralleled by the progressive increase of extracellular Ca^{2+} level. However, in line with other studies, the EPSP amplitude decreased abruptly at the onset of the on-state (Léger et al., 2005; Petersen et al., 2003; Sachdev et al., 2004). The underlying mechanisms for such increased excitability during the off-state may be related to passive membrane properties: The hyperpolarized membrane during the off-state increases the driving force for glutamatergic excitation, decreases the driving force for GABAergic inhibition, and increases input resistance (Crochet et al., 2005; Léger et al., 2005; Petersen et al., 2003; Sachdev et al., 2004). In line with these observation on the cellular level, Massimini and co-authors showed that the EEG amplitude of the cortical evoked response to sensory stimuli is altered in response to the sleep slow waves cycle

in humans (Massimini et al., 2003). The evoked response increased after the onset of the EEG negative slope and rose progressively until the onset of the positive EEG trend, followed by a continuous reduction during the subsequent up-phase of the slow wave. Taken together, the off-state during the slow oscillation, notably towards the end of the off-state, may provide a vulnerable time window to interfere with ongoing neuronal activity. Sensory information from the periphery reaches the cortex even during slow wave sleep (Issa and Wang, 2008; Massimini et al., 2003; Portas et al., 2000). Among the different somatosensory modalities, the auditory system efficaciously evokes a cortical response (*i.e*. presumably by activating non-lemniscal pathways of the sensory auditory system; Bellesi et al., 2014; Riedner et al., 2011; Tononi et al., 2010). Thus, auditory input delivered time-locked to the off-state of cortical neurons might, through the thalamus, terminate the off-state prematurly, thereby interfering with the ongoing highly synchronized activity of the neuronal network. Consequently, SWA will be reduced. The core question is now: What are the biological consequences of such SWA reduction?

5.3.3 The consequences of local slow wave manipulation

Slow waves during NREMS are supposed to be causally involved in the homeostatic regulation of cortical synapses (Tononi and Cirelli, 2014) and neurons (Vyazovskiy and Harris, 2013), to restore the brain's capacity to learn efficiently. To test this notion in humans, we performed a study, combining local slow wave perturbation with TMS and performance measures to assess the recuperative function of sleep (research article 6). We observed that selective slow wave perturbation in the left primary motor cortex causally prevented both, the learning induced increase in corticomotor excitability the next morning and the post sleep improvement of tapping variability. The stronger SWA was reduced the less recovery took place. These results provide first direct evidence for a causal role of sleep slow waves on the regulation of synaptic strength. As mentioned above, phase-timing analysis revealed that local SWA reduction was most successful, if tones were delivered after the negative peak (Figure 5). This observation concurs with the finding of Massimini et al. (2003). They found the strongest sensory cortical evoked response around the transition from the negative to the positive slope of slow waves. This results indicate that cortical neurons are most excitable at the transition from the off to the on-state of the underlying slow oscillation (Massimini et al., 2003). Thus, as highlighted above, the likelihood to provoke an abrupt termination of the off-state by auditory stimulation may be highest towards the end of the off-state of the slow oscillation. The progressive rise of extracellular Ca^{2+} concentration in the course of the off-state might restore synaptic efficacy, thereby increasing the probability of successful synaptic transmissions (Crochet et al., 2005; Massimini and Amzica, 2001). In line with this suggestion we found that auditory stimulation time-locked to the down-phase of slow waves over left primary motor cortex increased the following rising slope, and reduced the duration of the detected slow waves. Thus, auditory stimulation may have induced an abrupt transition from the underlying vulnerable off-state to the next on-state and thereby interfered with the ongoing highly synchronized activity of the neuronal network. As a result, the off-state which is thought to provide neurons with a time window to perform important restorative functions of cellular maintenance (Vyazovskiy and Harris, 2013) is shortened and the synchronized neuronal network activity, which is thought to reduce synaptic strength (Tononi and Cirelli, 2014), is altered. This may disturb the recuperative function of sleep slow waves on cellular and synaptic homeostasis.

Remarkably, a local reduction of SWA of ~10% was strong enough to induce measurable consequences on the behavioural and neuronal level. Nevertheless, this result does not show that local slow wave manipulation directly interfere with the homeostatic process of sleep. If so, one would expect that local slow wave disturbance during the first half of the night should be compensated by a rebound (*i.e*. locally increased SWA) in the second half of the night. First evidence for such a homeostatic rebound due to slow wave manipulation comes from the study presented by Santostasi and co-authors (2016). They applied auditory stimulation to enhance slow waves (*i.e*. by auditory stimulation time-locked to the up-phase of slow waves). In this study, auditory stimulation followed an alternating pattern of blocks with tones (on-blocks) and blocks without tones (off-blocks) and was compared to a non-stimulation condition. The authors found that SWA during on-blocks was increased compared to off-blocks and the non-stimulation condition. However, SWA during the off-blocks was not only reduced compared to the on-blocks, but also compared to the non-stimulation condition. Thus, increased SWA due to auditory stimulation during the on-blocks, was followed by a compensatory reduction of SWA during the off-blocks which fall below normal SWA values (Santostasi et al., 2016).

Unfortunately, our study design did not allow to investigate this question in more detail, since auditory stimulation was conducted throughout the entire night. Usually, due to the homeostatic process of sleep, the amount of stimulation (*i.e*. corresponding to the number of detected slow waves) was highest at the beginning of the night and decreased over the course of sleep, which makes a direct comparison between STIM and NOSTIM condition for early and late sleep difficult. However, in two subjects, a similar amount of auditory stimulation was delivered during early sleep (*i.e*. first and second sleep cycle), and late sleep (*i.e*. second las and last sleep cycle). In a preliminary analysis, SWA between STIM and NOSTIM condition during early and late sleep were compared in these two subjects (Figure 6). In both subjects, auditory stimulation during early sleep seemed to induce a rather global reduction of SWA (Figure 6 upper line). In contrast, during late sleep, SWA seemed to be globally increased, except for the brain region close to the stimulation electrode (Figure 6 middle line). Thus, if the entire night was considered, a local reduction of SWA close to the stimulating electrode can be observed in the STIM compared to the NOSTIM condition (Figure 6 bottom line).

Hence, during early sleep, when sleep pressure is high, the majority of slow waves may occur globally (Vyazovskiy and Harris, 2013; Vyazovskiy et al., 2011, 2009). As a consequent, during early sleep, auditory stimulation might be time-locked to the down-phase of slow waves in most brain areas, which results in a global decrease of SWA in the STIM compared to the NOSTIM condition. However, during late sleep, when sleep pressure has dissipated, slow waves appear more locally (Vyazovskiy and Harris, 2013; Vyazovskiy et al., 2011, 2009). Consequently, auditory stimulation time-locked to the down-phase of the detected slow waves becomes spatially more specific to the stimulation area. Thus, in the remaining brain, reduced SWA, due to auditory stimulation during early sleep, might be compensated during late sleep, which is reflected in a global increase of SWA compared to the NONSTIM condition. However, as auditory stimulation over the stimulation electrode persists to occur time-locked to the down-phase of sleep slow waves, SWA cannot be compensated over the stimulation electrode, resulting in a local decrease of SWA, if the entire night is considered. However, this interpretation is highly

speculative and only based on the results of two subjects. Thus, future studies are needed to shed further light on the underlying mechanism of local slow wave reduction and the role of local slow waves on the homeostatic process of sleep.

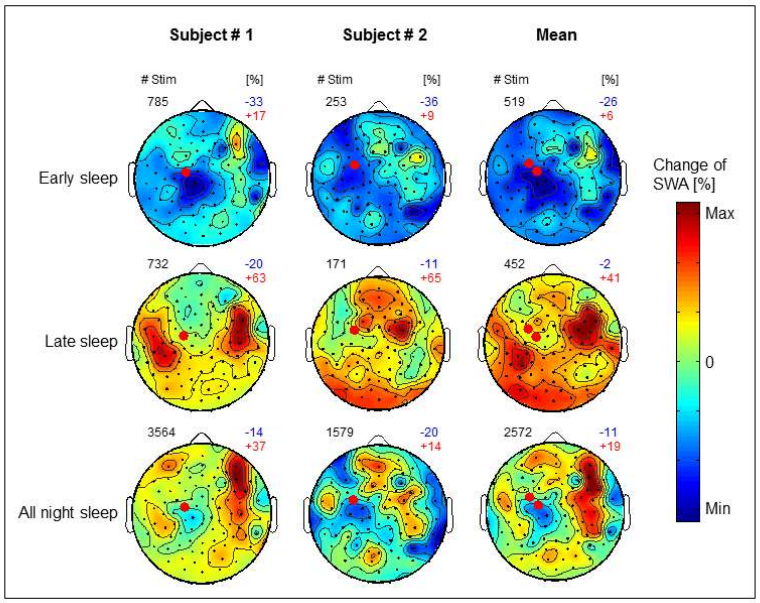

Figure 6

Percental topographical changes of SWA (1-2 Hz) between STIM and NOSTIM for early sleep (upper line), late sleep (middle line) and all night sleep (bottom line). Left and middle row: data from two single subjects, right row: mean values of these two single subjects. Blue colours indicate a decrease and red colour an increase in STIM compare to NOSTIM condition. Numbers in the upper right corners show maximal and minimal changes. Stimulation was triggered by slow waves detected over left motor cortex (*i.e*. stimulation electrode, indicated by a red dot). The number of stimulations for early, late and all night sleep are shown in the upper left corners. Note: stimulation seems to globally reduce SWA during early sleep, whereas during late sleep, SWA seems to be globally increased except around the stimulation electrode. When the entire night is considered, SWA was locally reduced around the stimulation electrode during STIM compared to NOSTIM (STIM = stimulation night; NOSTIM = non-stimulation night; data not published).

Yet, other important questions concerning the local specificity of slow wave perturbation require further investigations. On the one hand, the question arises whether SWA can be locally reduced in every brain area. For instance, local SWA reduction over frontal cortex might be more difficult, since the majority of slow waves originate over frontal regions and travel across the cortex (Massimini et al., 2004). Thus, by interfering with ongoing slow waves over frontal cortex, also other brain areas might be affected. Some support for this suggestion comes from a pilot recording, in which auditory stimulation was triggered by slow waves over left frontal cortex (Figure 4A upper example). Even though SWA reduction was most pronounced around the stimulation electrode, a general reduction of SWA over more distant brain areas was observed. On the other hand, the exact phase-timing of tone onset relative to the slow wave cycle to reduce SWA might differ between brain regions. In fact, preliminary results of phase-timing analysis for SWA reduction over right parietal cortex (including 7 subjects of the control experiment presented in

research article 6) point in this direction. In contrast to primary motor cortex, the analysis revealed that over right parietal cortex local SWA decrease was most successful, if the majority of the tones were applied shortly before the negative peak of the slow wave (*i.e*. section 3 in Figure 5B), however the correlation was not statistically significant. Hence, involvement of auditory sensory pathways (*i.e*. primary and associated pathways) might be differentially important to reach specific brain areas, and thus, different phase targeting may be required, depending on the location of SWA perturbation.

Other studies have successfully demonstrated that auditory stimulation time-locked to the up-phase of slow waves enhances SWA and improves retention of declarative memory (Ngo et al., 2013, 2015). Thus, during one pilot recording, we applied auditory stimulation with different time delays (*i.e*. 0 ms, 50 ms, and 200 ms) after slow wave detection, over left primary motor cortex (*i.e*. amplitude threshold of -30 µV). For each time delay, a similar number of stimuli was applied. They were randomly selected throughout the entire night. After each tone delivery, the online detection algorithm paused for 2 s, to allow offline exploration of the auditory event related potentials. As shown in Figure 7A, tones delivered with a time delay of 200 ms after slow wave detection (*i.e*. EEG signal reaches a negative amplitude of -30 µV) induced a higher amplitude wave compared to tones delivered with 0 ms or 50 ms delays. Phase-timing analysis revealed that the majority of the tones with a time delay of 200 ms were presented during the rising slope, just before the EEG signal crosses the zero-line (Figure 7D). Presumably, this reflects the underlying onset of the on-state. Thus, in line with other studies (Ngo et al., 2013, 2015; Santostasi et al., 2016), auditory stimulation during the active on-state of the slow oscillation may induce a fast and efficient synchronization of neuronal activity and thereby increase neuronal spiking. In turn, such increased activation during the on-state might result in pronounced neuronal hyperpolarization *i.e*. due to the activity-dependent nature of the I_{DK}, (for more details see section 3.1.1). This may be reflected in enhanced SWA on the EEG level (Bellesi et al., 2014). Such enhancement of neuronal activity due to incoming sensory stimuli may be most efficient at the beginning of the on-state when synaptic transmission is highest (Massimini and Amzica, 2001).

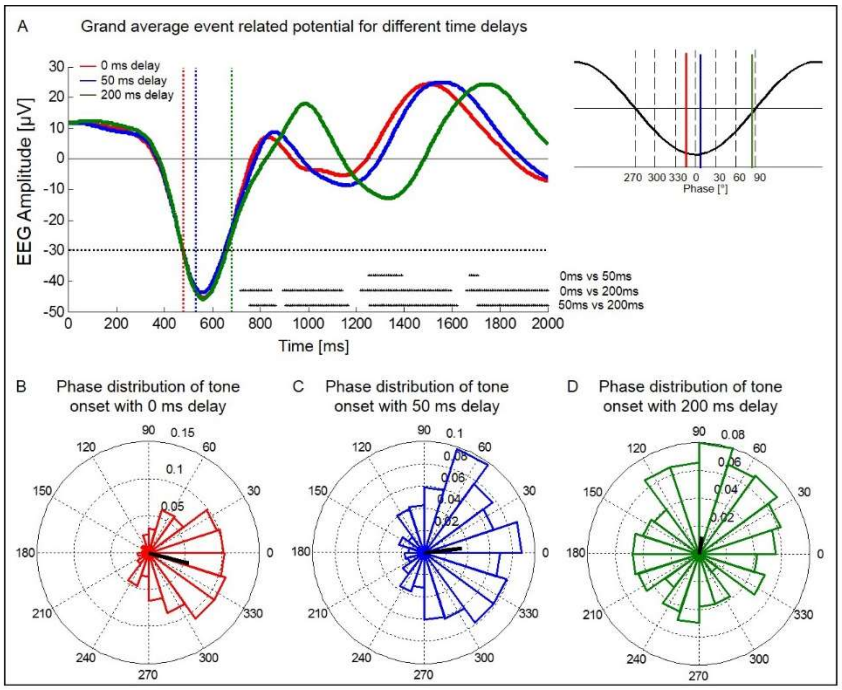

Figure 7

Example of one subject in which tones were delivered with different time delays after negative amplitude detection over left primary motor cortex. (A) Grand average event related potentials of the filtered EEG signal (0.5-2Hz) for different time delays, locked to the negative peak of the detected slow wave (*i.e*. at a negative amplitude of -30µV indicated by the black dashed line). Vertical dashed lines indicate the time point of tone onset (*i.e*. red = 0 ms, blue = 50 ms and green = 200 ms). Statistically different time points between the different time delays are indicated by black circles ($p<0.05$, different comparisons are indicated in the bottom right corner). Note: Tones delivered with a time delay of 200 ms induced a higher amplitude wave. Insert in the upper right corner: Schematic illustration of the different phase angles of the negative phase of the slow wave. Mean phase vector of tone onset for each time delay from Figure B-D are indicated (red = 0ms, blue = 50 ms and green = 200 ms). (B-D): Distribution of tone onset relative to the phase of the slow wave cycle for a time delay of 0ms (B), 50 ms (C) and 200 ms (D). The black lines represent the mean phase vectors. (Analysis was performed with the CircStat MatLab toolbox; Berens, 2009, data not published).

Our approach (*i.e*. introducing different time delays) to explore the induced auditory evoked response in relation to different phases of the slow wave cycle has some important limitations. First, phase targeting was rather imprecise (see Figure 7 B-D) and second, the direct comparison to a non-stimulation condition is missing. Other studies which focused on SWA enhancement by auditory stimulation have applied more accurate online detection algorithms, which take into account information of the phase of the ongoing EEG signal into account (Ngo et al., 2013, 2015; Santostasi et al., 2016). These studies have demonstrated that SWA can be successfully enhanced by auditory stimulation time-locked close to the positive peak of the ongoing slow wave. However, none of these studies has explored in detail for which phase of the slow wave cycle, the enhancement or reduction of SWA by auditory stimulation might be most efficient. Moreover, it has not been investigated how targeting different phases might be different in different brain areas. Thus, future studies are needed for a comprehensive understanding of SWA manipulation by auditory (or also other somatosensory modalities) stimulation. For example, the combination of hd-EEG recording with the above introduced PLL approach for online slow wave

detection (Santostasi et al., 2016) might allow a detailed phase targeting exploration of the entire slow wave cycle. Also, the induced consequences for the ongoing slow waves by sensory stimulation could be explored (like the analysis presented in Figure 7). Once the different parameters for the most efficient slow wave manipulation of different brain areas are established, such closed-loop sensory stimulation tools might provide a powerful tool to manipulate deep sleep locally and specifically (*i.e*. either increase of decrease SWA) in a non-invasive, simple, natural manner. Thus, such tools would allow to follow up on further important questions regarding the homeostatic function of sleep slow waves in humans. For example, what are the consequences of specific slow wave manipulation in the long term, *i.e*. if slow waves were not only manipulated during one night, but during several subsequent nights (*e.g*. during development, when a lot of changes in cortical connectivity occur)? Could such manipulations also be applied to theta waves in the wake EEG? Furthermore, also other physiological functions which have been related to sleep slow waves could be explored. For example, the regulation of blood pressure (Sayk et al., 2010), glucose and growth hormone secretion (Van Cauter, 2000) or memory consolidation (Diekelmann and Born, 2010; Tononi and Cirelli, 2014).

To sum up, in this thesis project, we quantified different indirect markers in the human surface EEG to track changes of neuronal activity related to the sleep-wake history. According to SHY, these changes reflect the sleep and wake-dependent regulation of synaptic strength. In fact, basic research on the molecular, synaptic, and cellular level has built a large body of evidence that synaptic regulation is linked to sleep slow waves (for review see Tononi and Cirelli, 2014). However, the human EEG, *i.e*. the viewpoint from the surface of the scalp, provides only an indirect measure of brain activity. Thus, relating different EEG characteristics to regulatory processes on the neuronal and synaptic level, remains speculative. Nevertheless, our results might provide one further piece of evidence that sleep plays an active role in the regulation of synaptic and neuronal homeostasis. All analysis presented in this thesis are based on human EEG recordings. Thus, these results were mainly interpreted in the framework of two hypothesis representing a corticocentric view of the function of sleep: SHY and sleep for the single neuron link the regulatory process of sleep to the homeostasis of cortical neurons and synapses. However, other theories for the function of sleep exist. They take a more generalized view, including recuperative processes for the brain *and* body (Adam, 1980; Berger and Philips, 1993). Furthermore, the role of REM sleep and the circadian process C were not considered in this thesis. During the years of my PhD project, I got to know many different facets of sleep and various approaches to investigate the role of these different aspects. I am convinced that sleep is vital and serves important biological functions. As stated by the famous sleep researcher and pioneer Allen Rechtschaffen: "*if sleep does not serve an absolute vital function, then it is the biggest mistake the evolutionary process ever made*." (Rechtschaffen, 1971). However, instead of looking for *the* fundamental function of sleep, I would rather keep open minded for the existence of various important roles of sleep among different species.

5.4 Concluding remarks and outlook

During the last decades, a large body of basic research, on the synaptic, single cell, network and computational level has built a translational bridge to relate different features in the EEG to the underlying neuronal activity patterns. These assumptions allow to track changes of neuronal network

activity related to the sleep-wake history, in humans non-invasively in the surface EEG. In the first part of the current thesis project, we have applied these recent findings from basic research to human wake and sleep EEG recordings during healthy and pathological development, a time window when the function of sleep slow waves in the regulation of synaptic strength might be particularly important (Ringli and Huber, 2011). In the second part of this work, we developed a closed-loop auditory stimulation tool, which allows to selectively manipulate slow waves focally, in a non-invasive natural manner. Thus, in future studies, such tools could be used to investigate the active role of sleep slow waves in shaping the morphological development of the human brain. Moreover, as such, this simple and non-invasive approach allowing long-term stimulation, might be ideally suited for clinical translation. As highlighted above, functional brain maturation is linked to morphological maturation. Thus, several mental and neurological disorders which have been related to altered regulatory processes of sleep, occur during development. Thereby, some of these altered processes were related to local changes in SWA. For example, compared to healthy control groups, SWA was locally increased over motor sensory brain areas in children diagnosed with ADHD (Ringli et al., 2013) and over frontal brain areas in children diagnosed with early onset depression (Tesler et al., 2016). Hence, in this patient groups, closed-loop auditory stimulation, which allows to selectively manipulate sleep slow waves in one specific brain area could serve as an ideal treatment tool. Yet another patient group which might benefit from such an approach are children diagnosed with CSWS. As described above, pathologically increased neuronal synchrony may contribute to the generation of spike wave activity in these patients. Assuming that auditory stimulation time-locked to the vulnerable off-state of cortical neurons may decrease network synchronization, such stimulation could reduce spike wave generation. To sum up, in the future basic research and different patient groups could benefit from such a closed-loop stimulation tool: It allows to (1) establish causal relationships, (2) target selective brain area, (3) it takes advantage of sleep as a time window for treatment and (4) it should not negatively affect daytime performance, since it does not interfere with the global structure of sleep.

6. References

Abásolo, D., Escudero, J., Hornero, R., Gómez, C., Espino, P., 2008. Approximate Entropy and auto mutual information analysis of the electroencephalogram in {Alzheimer's} disease patients. Med. Biol. Eng. Comput. 46, 1019–1028. doi:10.1007/s11517-008-0392-1

Achermann, P., Borbély, A.A., 2003. Mathematical models of sleep regulation. Front Biosci 8, s683–93.

Achermann, P., Borbély, A.A., 1997. Low-frequency (< 1 hz) oscillations in the human sleep electroencephalogram. Neuroscience 81, 213–222. doi:10.1016/S0306-4522(97)00186-3

Adam, K., 1980. Sleep as a restorative process and a theory to explain why. Prog. Brain Res. 53, 289–305. doi:10.1016/S0079-6123(08)60070-9

Aeby, A., Poznanski, N., Verheulpen, D., Wetzburger, C., Van Bogaert, P., 2005. Levetiracetam efficacy in epileptic syndromes with continuous spikes and waves during slow sleep: experience in 12 cases. Epilepsia 46, 1937–42. doi:10.1111/j.1528-1167.2005.00337.x

Aeschbach, D., Cutler, A.J., Ronda, J.M., 2008. A role for non-rapid-eye-movement sleep homeostasis in perceptual learning. J. Neurosci. 28, 2766–72. doi:10.1523/JNEUROSCI.5548-07.2008

Aeschbach, D., Dijk, D.J., Borbely, A.A., 1997a. Dynamics of EEG spindle frequency activity during extended sleep in humans: relationship to slow-wave activity and time of day. Brain Res 748, 131–136. doi:S0006-8993(96)01275-9 [pii]

Aeschbach, D., Matthews, J.R., Postolache, T.T., Jackson, M.A., Giesen, H.A., Wehr, T.A., 1999. Two circadian rhythms in the human electroencephalogram during wakefulness. Am. J. Physiol. 277, R1771–9.

Aeschbach, D., Matthews, J.R., Postolache, T.T., Jackson, M.A., Giesen, H.A., Wehr, T.A., 1997b. Dynamics of the human EEG during prolonged wakefulness: evidence for frequency-specific circadian and homeostatic influences. Neurosci. Lett. 239, 121–124. doi:S0304-3940(97)00904-X [pii]

Akerstedt, T., Gillberg, M., 1990. Subjective and objective sleepiness in the active individual. Int. J. Neurosci. 52, 29–37.

Alfoldi, P., Tobler, I., Borbely, A.A., 1990. Sleep regulation in rats during early development. Am J Physiol 258, R634–44.

Allen, D.G., Lamb, G.D., Westerblad, H., 2008. Skeletal Muscle Fatigue: Cellular Mechanisms. Physiol. Rev. 88.

Anders, T., Emde, R., Parmelee, A., 1971. A manual of standardized terminology, techniques and criteria for scoring of states of sleep and wakefulness in newborn infants . UCLA Brain Information Service/Brain Research Institute, Los Angesles, CA.

Anders, T.F., Keener, M., 1985. Developmental course of nighttime sleep-wake patterns in full-term and premature infants during the first year of life. I. Sleep 8, 173–192.

Anier, A., Lipping, T., Ferenets, R., Puumala, P., Sonkajärvi, E., Rätsep, I., Jäntti, V., 2012. Relationship between approximate entropy and visual inspection of irregularity in the {EEG} signal, a comparison with spectral entropy. Br. J. Anaesth. 109, 928–934. doi:10.1093/bja/aes312

Arzi, A., Shedlesky, L., Ben-Shaul, M., Nasser, K., Oksenberg, A., Hairston, I.S., Sobel, N., 2012. Humans can learn new information during sleep. Nat. Neurosci. 15, 1460–5.

doi:10.1038/nn.3193

Balduzzi, D., Tononi, G., 2008. Integrated Information in Discrete Dynamical Systems: {Motivation} and Theoretical Framework. PLoS Comput. Biol. 4, e1000091. doi:10.1371/journal.pcbi.1000091

Bastien, C.H., Crowley, K.E., Colrain, I.M., 2002. Evoked potential components unique to non-REM sleep: relationship to evoked K-complexes and vertex sharp waves. Int. J. Psychophysiol. 46, 257–274. doi:10.1016/S0167-8760(02)00117-4

Baumbach, H.D., 1981. Visuocortical epileptiform discharges in rabbits: Differential effects on neuronal development in the lateral geniculate nucleus and superior colliculus. Brain Res. 209, 61–76. doi:10.1016/0006-8993(81)91172-0

Bazhenov, M., Timofeev, I., Steriade, M., Sejnowski, T.J., 2002. Model of thalamocortical slow-wave sleep oscillations and transitions to activated States. J Neurosci 22, 8691–8704. doi:22/19/8691 [pii]

Bellesi, M., Riedner, B.A., Garcia-Molina, G.N., Cirelli, C., Tononi, G., 2014. Enhancement of sleep slow waves: underlying mechanisms and practical consequences. Front Syst Neurosci 8, 208. doi:10.3389/fnsys.2014.00208

Bentson, J., Reza, M., Winter, J., Wilson, G., 1978. Steroids and apparent cerebral atrophy on computed tomography scans. J Comput Assist Tomogr 2, 16–23.

Berens, P., 2009. CircStat: A Matlab Toolbox for Circular Statistics. J. Stat. Softw. 31.

Berg, A.T., Millichap, J.J., 2013. The 2010 revised classification of seizures and epilepsy. Contin. (Minneap Minn) 19, 571–597. doi:10.1212/01.CON.0000431377.44312.9e00132979-201306000-00008 [pii]

Berger, H., 1929. Über das elektrenkephalogramm des menschen. Eur Arch Psy Clin N 87, 527.

Berger, R., Philips, N., 1993. Sleep and Energy Conservation. News Physiol Sci 8, 276–281.

Bersagliere, A., Achermann, P., 2010. Slow oscillations in human non-rapid eye movement sleep electroencephalogram: effects of increased sleep pressure. J. Sleep Res. 19, 228–237. doi:DOI 10.1111/j.1365-2869.2009.00775.x

Bes, F., Schulz, H., Navelet, Y., Salzarulo, P., 1991. The distribution of slow-wave sleep across the night: a comparison for infants, children, and adults. Sleep 14, 5–12.

Bestmann, S., Krakauer, J.W., 2015. The uses and interpretations of the motor-evoked potential for understanding behaviour. Exp. Brain Res. 233, 679–689. doi:10.1007/s00221-014-4183-7

Blake, H., Gerard, R., Kleitman, N., 1939. FACTORS INFLUENCING BRAIN POTENTIALS DURING SLEEP. J. Neurophysiol. 2.

Bölsterli, B.K., Schmitt, B., Bast, T., Critelli, H., Heinzle, J., Jenni, O.G., Huber, R., Bolsterli, B.K., Schmitt, B., Bast, T., Critelli, H., Heinzle, J., Jenni, O.G., Huber, R., Bölsterli, B.K., Schmitt, B., Bast, T., Critelli, H., Heinzle, J., Jenni, O.G., Huber, R., Bolsterli, B.K., Schmitt, B., Bast, T., Critelli, H., Heinzle, J., Jenni, O.G., Huber, R., 2011. Impaired slow wave sleep downscaling in encephalopathy with status epilepticus during sleep (ESES). Clin. Neurophysiol. 122, 1779–1787. doi:DOI 10.1016/j.clinph.2011.01.053

Bolsterli Heinzle, B.K., Fattinger, S., Kurth, S., Lebourgeois, M.K., Ringli, M., Bast, T., Critelli, H., Schmitt, B., Huber, R., 2014. Spike wave location and density disturb sleep slow waves in patients with CSWS (continuous spike waves during sleep). Epilepsia 55, 584–591. doi:10.1111/epi.12576

Borbély, A.A., 1982. A two process model of sleep regulation. Hum. Neurobiol. 1, 195–204.

Borbély, A.A., Achermann, P., 2011. Homeostasis of human sleep and models of sleep regulation., in: Kryger, M.H., Roth, T., Dement, W.C. (Eds.), Principles and Practice of Sleep Medicine. W. B. Saunders, Philadelphia, pp. 377–390.

Borbely, A.A., Baumann, F., Brandeis, D., Strauch, I., Lehmann, D., 1981. Sleep deprivation: effect on sleep stages and EEG power density in man. Electroencephalogr Clin Neurophysiol 51, 483–495.

Borbely, A.A., Tobler, I., Hanagasioglu, M., 1984. Effect of sleep deprivation on sleep and EEG power spectra in the rat. Behav Brain Res 14, 171–182.

Brown, S.M., Henning, S., Wellman, C.L., 2005. Mild, short-term stress alters dendritic morphology in rat medial prefrontal cortex. Cereb Cortex 15, 1714–1722. doi:bhi048 [pii]10.1093/cercor/bhi048

Bruhn, J., Bouillon, T.W., Radulescu, L., Hoeft, A., Bertaccini, E., Shafer, S.L., 2003. Correlation of Approximate Entropy, Bispectral Index, and Spectral Edge Frequency 95 {(SEF95)} with Clinical Signs of “Anesthetic Depth” during Coadministration of Propofol and Remifentanil. Anesthesiology 98, 621–627.

Bruhn, J., Röpcke, H., Hoeft, A., 2000a. Approximate Entropy as an Electroencephalographic Measure of Anesthetic Drug Effect during Desflurane Anesthesia. Anesthesiology 92, 715–726.

Bruhn, J., Röpcke, H., Rehberg, B., Bouillon, T., Hoeft, A., 2000b. Electroencephalogram Approximate Entropy Correctly Classifies the Occurrence of Burst Suppression Pattern as Increasing Anesthetic Drug Effect. Anesthesiology 93, 981–985.

Buchmann, A., Ringli, M., Kurth, S., Schaerer, M., Geiger, A., Jenni, O.G., Huber, R., 2010. EEG Sleep Slow-Wave Activity as a Mirror of Cortical Maturation. Cereb Cortex 21, 607–615. doi:bhq129 [pii]10.1093/cercor/bhq129

Burioka, N., Miyata, M., Cornelissen, G., Halberg, F., Takeshima, T., Kaplan, D.T., Suyama, H., Endo, M., Maegaki, Y., Nomura, T., Tomita, Y., Nakashima, K., Shimizu, E., 2005. Approximate Entropy in the Electroencephalogram during Wake and Sleep. Clin. EEG Neurosci. 36, 21–24. doi:10.1177/155005940503600106

Burré, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M.R., Südhof, T.C., 2010. α-Synuclein Promotes SNARE-Complex Assembly in Vivo and in Vitro. Science (80-.). 329.

Bushey, D., Tononi, G., Cirelli, C., 2011. Sleep and synaptic homeostasis: structural evidence in Drosophila. Science (80-.). 332, 1576–1581. doi:332/6037/1576 [pii]10.1126/science.1202839

Butefisch, C.M., Davis, B.C., Wise, S.P., Sawaki, L., Kopylev, L., Classen, J., Cohen, L.G., 2000. Mechanisms of use-dependent plasticity in the human motor cortex. Proc. Natl. Acad. Sci. 97, 3661–3665. doi:10.1073/pnas.97.7.3661

Buzsaki, G., 2006. Rhythms of the Brain. Oxford University Press, New York, NY.

Buzsáki, G., 1989. Two-stage model of memory trace formation: A role for “noisy” brain states. Neuroscience 31, 551–570. doi:10.1016/0306-4522(89)90423-5

C. Hanlon, E., V. Vyazovskiy, V., Faraguna, U., Tononi, G., Cirelli, C., 2011. Synaptic Potentiation and Sleep Need: Clues from Molecular and Electrophysiological Studies. Curr. Top. Med. Chem. 11, 2472–2482. doi:10.2174/156802611797470312

Cajochen, C., Brunner, D.P., Krauchi, K., Graw, P., Wirz-Justice, A., 1995. Power density in theta/alpha frequencies of the waking EEG progressively increases during sustained wakefulness. Sleep 18, 890–894.

Cajochen, C., Khalsa, S.B.S., Wyatt, J.K., Czeisler, C.A., Dijk, D.J., 1999. EEG and ocular correlates

of circadian melatonin phase and human performance decrements during sleep loss. Am. J. Physiol. Integr. Comp. Physiol. 277, R640–R649.

Cajochen, C., Knoblauch, V., Krauchi, K., Renz, C., Wirz-Justice, A., 2001. Dynamics of frontal EEG activity, sleepiness and body temperature under high and low sleep pressure. Neuroreport 12, 2277–2281.

Cajochen, C., Wyatt, J.K., Czeisler, C.A., Dijk, D.J., 2002. Separation of circadian and wake duration-dependent modulation of EEG activation during wakefulness. Neuroscience 114, 1047–1060. doi:S0306452202002099 [pii]

Campbell, I.G., Feinberg, I., 2009. Longitudinal trajectories of non-rapid eye movement delta and theta EEG as indicators of adolescent brain maturation. Proc Natl Acad Sci U S A 106, 5177–5180. doi:0812947106 [pii]10.1073/pnas.0812947106

Campbell, S.S., Tobler, I., 1984. Animal sleep: a review of sleep duration across phylogeny. Neurosci. Biobehav. Rev. 8, 269–300.

Caraballo, R.H., Veggiotti, P., Kaltenmeier, M.C., Piazza, E., Gamboni, B., Lopez Avaria, M.F., Noli, D., Adi, J., Cersosimo, R., 2013. Encephalopathy with status epilepticus during sleep or continuous spikes and waves during slow sleep syndrome: a multicenter, long-term follow-up study of 117 patients. Epilepsy Res. 105, 164–73. doi:10.1016/j.eplepsyres.2013.02.010

Carskadon, M.A., Acebo, C., 2002. Regulation of sleepiness in adolescents: Update, insights, and speculation. Sleep 25, 606–614.

Carskadon, M.A., Acebo, C., Jenni, O.G., 2004. Regulation of adolescent sleep: implications for behavior. Ann NY Acad Sci 1021, 276–291. doi:10.1196/annals.1308.032

Carskadon, M.A., Dement, W.C., 2011. Normal Human Sleep : An Overview. Princ. Pract. sleep Med. 16–26. doi:10.1016/B978-1-4160-6645-3.00141-9

Carskadon, M.A., Harvey, K., Duke, P., Anders, T.F., Litt, I.F., Dement, W.C., 1980. Pubertal changes in daytime sleepiness. Sleep 2, 453–460.

Carson, R.G., Nelson, B.D., Buick, A.R., Carroll, T.J., Kennedy, N.C., Cann, R. Mac, 2013. Characterizing changes in the excitability of corticospinal projections to proximal muscles of the upper limb. Brain Stimul. 6, 760–8. doi:10.1016/j.brs.2013.01.016

Carson, R.G., Ruddy, K.L., Mc Nickle, E., 2016. What do TMS evoked motor potentials tell us about motor learning?, Progress in Motor Control; Theories and Translations. Springer International Publishing. doi:10.1007/7854

Casali, A.G., Gosseries, O., Rosanova, M., Boly, M., Sarasso, S., Casali, K.R., Casarotto, S., Bruno, M.-A., Laureys, S., Tononi, G., Massimini, M., 2013. A Theoretically Based Index of Consciousness Independent of Sensory Processing and Behavior. Sci. Transl. Med. 5, 198ra105. doi:10.1126/scitranslmed.3006294

Chauvette, S., Volgushev, M., Timofeev, I., 2010. Origin of active states in local neocortical networks during slow sleep oscillation. Cereb Cortex 20, 2660–2674. doi:bhq009 [pii]10.1093/cercor/bhq009

Cirelli, C., Gutierrez, C.M., Tononi, G., 2004. Extensive and Divergent Effects of Sleep and Wakefulness on Brain Gene Expression. Neuron 41, 35–43. doi:10.1016/S0896-6273(03)00814-6

Cirelli, C., Tononi, G., 2008. Is sleep essential? Plos Biol. 6, 1605–1611. doi:ARTN e216DOI 10.1371/journal.pbio.0060216

Classen, J., Liepert, J., Wise, S.P., Hallett, M., Leonardo, G., Baarbé, J., Yielder, P., Daligadu, J., Behbahani, H., Haavik, H., Candidi, M., Sacheli, L.M., Mega, I., Aglioti, S.M., Cassidy, J.M., Gillick, B.T., Carey, J.R., Cohen, L.G., 1998. Rapid Plasticity of Human Cortical Movement Representation Induced by Practice Rapid Plasticity of Human Cortical Movement Representation Induced by Practice. J. Neurophysiol. 1117–1123.

Colrain, I.M., 2005. The K-complex: a 7-decade history. Sleep 28, 255–73.

Contreras, D., Timofeev, I., Steriade, M., 1996. Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. J. Physiol. 494, 251–264. doi:10.1113/jphysiol.1996.sp021488

Cook, S.C., Wellman, C.L., 2004. Chronic stress alters dendritic morphology in rat medial prefrontal cortex. J Neurobiol 60, 236–248. doi:10.1002/neu.20025

Coons, S., Guilleminault, C., 1982. Development of sleep-wake patterns and non-rapid eye movement sleep stages during the first six months of life in normal infants. Pediatrics 69, 793–798.

Copinschi, G., Leproult, R., Spiegel, K., 2014. The important role of sleep in metabolism. Front. Horm. Res. 42, 59–72. doi:10.1159/000358858

Couyoumdjian, A., Sdoia, S., Tempesta, D., Curcio, G., Rastellini, E., L, D.E.G., Ferrara, M., 2010. The effects of sleep and sleep deprivation on task-switching performance. J Sleep Res 19, 64–70. doi:JSR774 [pii]10.1111/j.1365-2869.2009.00774.x

Cox, R., Korjoukov, I., de Boer, M., Talamini, L.M., 2014. Sound asleep: processing and retention of slow oscillation phase-targeted stimuli. PLoS One 9, e101567. doi:10.1371/journal.pone.0101567

Crochet, S., Chauvette, S., Boucetta, S., Timofeev, I., 2005. Modulation of synaptic transmission in neocortex by network activities. Eur. J. Neurosci. 21, 1030–44. doi:10.1111/j.1460-9568.2005.03932.x

Crochet, S., Petersen, C.C.H., 2006. Correlating whisker behavior with membrane potential in barrel cortex of awake mice. Nat. Neurosci. 9, 608–610. doi:10.1038/nn1690

Crunelli, V., Hughes, S.W., 2010. The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. Nat Neurosci 13, 9–17. doi:10.1038/nn.2445

Czarnecki, A., Birtoli, B., Ulrich, D., 2007. Cellular mechanisms of burst firing-mediated long-term depression in rat neocortical pyramidal cells. J Physiol 578, 471–479. doi:jphysiol.2006.123588 [pii]10.1113/jphysiol.2006.123588

Dalla Bernardina B, Watanabe, K., 1994. Interictal EEG: variations and pitfalls, in: Dulac O Dalla Bernardina B, C.H.T. (Ed.), Infantile Spasms and West Syndrome. W.B. Saunders Company LTD, London 1994; chapter 6: 63-81, pp. 63–81.

Dang-Vu, T.T., Schabus, M., Desseilles, M., Albouy, G., Boly, M., Darsaud, A., Gais, S., Rauchs, G., Sterpenich, V., Vandewalle, G., Carrier, J., Moonen, G., Balteau, E., Degueldre, C., Luxen, A., Phillips, C., Maquet, P., 2008. Spontaneous neural activity during human slow wave sleep. Proc. Natl. Acad. Sci. U. S. A. 105, 15160–5. doi:10.1073/pnas.0801819105

de Beukelaar, T., Soom, J., Huber, R., Wenderoth, N., 2016. Sleep restores the potential to undergo learning-induced increases incorticomotor excitability. Appl Erg.

de Beukelaar, T.T., Woolley, D.G., Wenderoth, N., 2014. Gone for 60 seconds: reactivation length determines motor memory degradation during reconsolidation. Cortex. 59, 138–45. doi:10.1016/j.cortex.2014.07.008

Delorme, A., Makeig, S., 2004. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J. Neurosci. Methods 134, 9–21. doi:10.1016/j.jneumeth.2003.10.009

Denker, A., Rizzoli, S.O., 2010. Synaptic vesicle pools: an update. Front. Synaptic Neurosci. 2, 135. doi:10.3389/fnsyn.2010.00135

Devanne, H., Lavoie, B.A., Capaday, C., 1997. Input-output properties and gain changes in the human corticospinal pathway. Exp. Brain Res. 114, 329–338. doi:10.1007/PL00005641

Diekelmann, S., Born, J., 2010. The memory function of sleep. Nat Rev Neurosci 11, 114–126. doi:nrn2762 [pii]10.1038/nrn2762

Dijk, D.J., Czeisler, C.A., 1995. Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. J Neurosci 15, 3526–3538.

Dijk, D.J., Duffy, J.F., Czeisler, C.A., 1992. Circadian and sleep/wake dependent aspects of subjective alertness and cognitive performance. J. Sleep Res. 1, 112–117. doi:jsr001002112 [pii]

Doran, S.M., Van Dongen, H.P.A., Dinges, D.F., 2001. Sustained attention performance during sleep deprivation: Evidence of state instability. Arch. Ital. Biol. 139, 253–267.

Driver, H.S., Dijk, D.J., Werth, E., Biedermann, K., Borbely, A.A., 1996. Sleep and the sleep electroencephalogram across the menstrual cycle in young healthy women. J. Clin. Endocrinol. Metab. 81, 728–735.

Dumont, M., Macchi, M.M., Carrier, J., Lafrance, C., Hebert, M., 1999. Time course of narrow frequency bands in the waking EEG during sleep deprivation. Neuroreport 10, 403–407.

Esser, S.K., Hill, S.L., Tononi, G., 2007. Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. Sleep 30, 1617–1630.

Esser, S.K., Huber, R., Massimini, M., Peterson, M.J., Ferrarelli, F., Tononi, G., 2006. A direct demonstration of cortical LTP in humans: a combined TMS/EEG study. Brain Res Bull 69, 86–94. doi:S0361-9230(05)00458-2 [pii]10.1016/j.brainresbull.2005.11.003

Fagioli, I., Salzarulo, P., 1982. Sleep states development in the first year of life assessed through 24-h recordings. Early Hum Dev 6, 215–228.

Fair, D. a, Cohen, A.L., Dosenbach, N.U.F., Church, J. a, Miezin, F.M., Barch, D.M., Raichle, M.E., Petersen, S.E., Schlaggar, B.L., 2008. The maturing architecture of the brain's default network. Proc. Natl. Acad. Sci. U. S. A. 105, 4028–4032. doi:10.1073/pnas.0800376105

Fair, D.A., Dosenbach, N.U.F., Church, J.A., Cohen, A.L., Brahmbhatt, S., Miezin, F.M., Barch, D.M., Raichle, M.E., Petersen, S.E., Schlaggar, B.L., Dosenbach, N.U.F., Church, J.A., Miezin, F.M., Barch, D.M., Raichle, M.E., Petersen, S.E., Schlaggar, B.L., Power, J.D., Dosenbach, N.U.F., Church, J.A., Miezin, F.M., Schlaggar, B.L., Petersen, S.E., 2007. Functional brain networks develop from a "local to distributed" organization. Proc. Natl. Acad. Sci. U. S. A. 5, 13507–12. doi:10.1073/pnas.0705843104

Fattinger, S., Jenni, O.G., Schmitt, B., Achermann, P., Huber, R., 2014. Overnight Changes in the Slope of Sleep Slow Waves during Infancy. Sleep 37, 245–253. doi:10.5665/sleep.3390

Fattinger, S., Schmitt, B., Bolsterli Heinzle, B.K., Critelli, H., Jenni, O.G., Huber, R., Bölsterli Heinzle, B.K., Critelli, H., Jenni, O.G., Huber, R., Bolsterli Heinzle, B.K., Critelli, H., Jenni, O.G., Huber, R., Bölsterli Heinzle, B.K., Critelli, H., Jenni, O.G., Huber, R., 2015. Impaired slow wave sleep downscaling in patients with infantile spasms. Eur J Paediatr Neurol 19, 134–142. doi:S1090-3798(14)00177-9 [pii]10.1016/j.ejpn.2014.11.002

Feinberg, I., 1982. Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? J Psychiatr Res 17, 319–334.

Feinberg, I., Campbell, I.G., 2010. Sleep EEG changes during adolescence: an index of a fundamental brain reorganization. Brain Cogn 72, 56–65. doi:S0278-2626(09)00179-1 [pii]10.1016/j.bandc.2009.09.008

Ferrarelli, F., Huber, R., Peterson, M.J., Massimini, M., Murphy, M., Riedner, B.A., Watson, A., Bria, P., Tononi, G., 2007. Reduced sleep spindle activity in schizophrenia patients. Am J Psychiatry 164, 483–492. doi:164/3/483 [pii]10.1176/appi.ajp.164.3.483

Ferrarelli, F., Massimini, M., Sarasso, S., Casali, A., Riedner, B.A., Angelini, G., Tononi, G., Pearce, R.A., 2010. Breakdown in cortical effective connectivity during midazolam-induced loss of consciousness. Proc. Natl. Acad. Sci. U.S.A. 107, 2681–2686. doi:10.1073/pnas.0913008107

Ferri, R., Rundo, F., Bruni, O., Terzano, M.G., Stam, C.J., 2008. The functional connectivity of different {EEG} bands moves towards small-world network organization during sleep. Clin. Neurophysiol. 119, 2026–2036. doi:10.1016/j.clinph.2008.04.294

Ferri, R., Rundo, F., Bruni, O., Terzano, M.G., Stam, C.J., 2007. Small-world network organization of functional connectivity of {EEG} slow-wave activity during sleep. Clin. Neurophysiol. 118, 449–456.

Finelli, L.A., Baumann, H., Borbely, A.A., Achermann, P., 2000. Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. Neuroscience 101, 523–529. doi:S0306-4522(00)00409-7 [pii]

Flores Vega, C.H., Noel, J., Fernández, J.R., 2013. Cognitive task discrimination using approximate entropy ({ApEn}) on {EEG} signals, in: Biosignals and Biorobotics Conference (BRC), 2013 ISSNIP. pp. 1–4. doi:10.1109/BRC.2013.6487521

Franken, P., Dijk, D.-J.J., 2009. Circadian clock genes and sleep homeostasis. Eur J Neurosci 29, 1820–1829. doi:EJN6723 [pii]10.1111/j.1460-9568.2009.06723.x

Frauscher, B., von Ellenrieder, N., Ferrari-Marinho, T., Avoli, M., Dubeau, F., Gotman, J., 2015. Facilitation of epileptic activity during sleep is mediated by high amplitude slow waves. Brain 138, 1629–41. doi:10.1093/brain/awv073

Gasser, T., Jennen-Steinmetz, C., Sroka, L., Verleger, R., Mocks, J., 1988. Development of the EEG of school-age children and adolescents. II. Topography. Electroencephalogr. Clin. Neurophysiol. 69, 100–109.

Gaudreau, H., Carrier, J., Montplaisir, J., 2001. Age-related modifications of NREM sleep EEG: from childhood to middle age. J. Sleep Res. 10, 165–172. doi:252 [pii]

Gelman, A., Hill, J., 2006. Data Analysis Using Regression and Multilevel/Hierarchical Models.

Gibson, J.R., Beierlein, M., Connors, B.W., 1999. Two networks of electrically coupled inhibitory neurons in neocortex. Nature 402, 75–9. doi:10.1038/47035

Giedd, J.N., 2004. Structural magnetic resonance imaging of the adolescent brain. Ann. N. Y. Acad. Sci. 1021, 77–85.

Gilestro, G.F., Tononi, G., Cirelli, C., 2009. Widespread changes in synaptic markers as a function of sleep and wakefulness in Drosophila. Science (80-.). 324, 109–112. doi:324/5923/109 [pii]10.1126/science.1166673

Gogtay, N., Giedd, J.N., Lusk, L., Hayashi, K.M., Greenstein, D., Vaituzis, A.C., Nugent 3rd, T.F., Herman, D.H., Clasen, L.S., Toga, A.W., Rapoport, J.L., Thompson, P.M., 2004. Dynamic

mapping of human cortical development during childhood through early adulthood. Proc Natl Acad Sci U S A 101, 8174–8179. doi:10.1073/pnas.0402680101010402680101 [pii]

Gordon, N., 1980. Apparent cerebral atrophy in patients on treatment with steroids. Dev Med Child Neurol 22, 502–506.

Gu, F., Meng, X., Shen, E., Cai, Z., 2003. Can We Measure Consciousness with {EEG} Complexities? Int. J. Bifurcat. Chaos 13, 733–742.

Gueorguieva, R., Krystal, J.H., 2004. Move Over ANOVA: Progress in Analyzing Repeated-Measures Data andIts Reflection in Papers Published in the Archives of General Psychiatry. Arch. Gen. Psychiatry 61, 310. doi:10.1001/archpsyc.61.3.310

Guilleminault, C., Soquet, M., 1978. Sleep states and related pathology, Advances in perinatal Neurology. SP Medical, New York.

Guyon, A., Balbo, M., Morselli, L.L., Tasali, E., Leproult, R., L'Hermite-Balériaux, M., Van Cauter, E., Spiegel, K., 2014. Adverse Effects of Two Nights of Sleep Restriction on the Hypothalamic-Pituitary-Adrenal Axis in Healthy Men. J. Clin. Endocrinol. Metab. 99, 2861–2868. doi:10.1210/jc.2013-4254

Hagenauer, M.H., Perryman, J.I., Lee, T.M., Carskadon, M.A., 2009. Adolescent Changes in the Homeostatic and Circadian Regulation of Sleep. Dev Neurosci 31, 276–284. doi:000216538 [pii]10.1159/000216538

Hancock, E.C., Osborne, J.P., Edwards, S.W., 2009. Treatment of infantile spasms. Cochrane Database Syst Rev CD001770.

Harris, K.D., Thiele, A., 2011. Cortical state and attention. Nat. Rev. Neurosci. 12, 509–523. doi:10.1038/nrn3084

Hayashi, K., Shigemi, K., Sawa, T., 2012. Neonatal electroencephalography shows low sensitivity to anesthesia. Neurosci. Lett. 517, 87–91. doi:10.1016/j.neulet.2012.04.028

Hess, C.W., Mills, K.R., Murray, N.M., Schriefer, T.N., 1987. Magnetic brain stimulation: central motor conduction studies in multiple sclerosis. Ann. Neurol. 22, 744–52. doi:10.1002/ana.410220611

Hill, S., Tononi, G., 2005. Modeling sleep and wakefulness in the thalamocortical system. J. Neurophysiol. 93, 1671–1698. doi:00915.2004 [pii]10.1152/jn.00915.2004

Hill, S., Tononi, G., Ghilardi, M.F., 2008. Sleep improves the variability of motor performance. Brain Res Bull 76, 605–611.

Hirshkowitz, M., 2004. Normal human sleep: an overview. Med. Clin. North Am. 88, 551–65, vii. doi:10.1016/j.mcna.2004.01.001

Hjorth, B., 1975. An on-line transformation of EEG scalp potentials into orthogonal source derivations. Electroencephalogr. Clin. Neurophysiol. 39, 526–530. doi:10.1016/0013-4694(75)90056-5

Holmes, G.L., Lenck-Santini, P.-P., 2006. Role of interictal epileptiform abnormalities in cognitive impairment. Epilepsy Behav. 8, 504–15. doi:10.1016/j.yebeh.2005.11.014

Holsboer, F., von Bardeleben, U., Steiger, A., 2008. Effects of Intravenous Corticotropin-Releasing Hormone upon Sleep-Related Growth Hormone Surge and Sleep EEG in Man. Neuroendocrinology 48, 32–38. doi:10.1159/000124986

Hrachovy, R.A., Frost Jr., J.D., Kellaway, P., 1981. Sleep characteristics in infantile spasms. Neurology 31, 688–693.

Huber, R., Ghilardi, M.F., Massimini, M., Ferrarelli, F., Riedner, B.A., Peterson, M.J., Tononi, G., 2006.

Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. Nat Neurosci 9, 1169–1176.

Huber, R., Ghilardi, M.F., Massimini, M., Tononi, G., 2004. Local sleep and learning. Nature 430, 78–81. doi:10.1038/nature02663nature02663 [pii]

Huber, R., Graf, T., Cote, K.A., Wittmann, L., Gallmann, E., Matter, D., Schuderer, J., Kuster, N., Borbely, A.A., Achermann, P., 2000. Exposure to pulsed high-frequency electromagnetic field during waking affects human sleep EEG. Neuroreport 11, 3321–3325.

Huber, R., Maki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A.G., Tononi, G., Massimini, M., 2013. Human cortical excitability increases with time awake. Cereb Cortex 23, 332–338. doi:bhs014 [pii]10.1093/cercor/bhs014

Huber, R., Tononi, G., Cirelli, C., 2007. Exploratory behavior, cortical BDNF expression, and sleep homeostasis. Sleep 30, 129–139.

Hulse, B.K., Landsness, E.C., Sarasso, S., Ferrarelli, F., Guokas, J.J., Wanger, T., Tononi, G., 2011. A postsleep decline in auditory evoked potential amplitude reflects sleep homeostasis. Clin. Neurophysiol. 122, 1549–1555. doi:S1388-2457(11)00091-5 [pii]10.1016/j.clinph.2011.01.041

Hung, C.-S., Sarasso, S., Ferrarelli, F., Riedner, B., Ghilardi, M.F., Cirelli, C., Tononi, G., 2013. Local Experience-Dependent Changes in the Wake EEG after Prolonged Wakefulness. Sleep 36, 59–72. doi:10.5665/sleep.2302

Huttenlocher, P.R., 1990. Morphometric study of human cerebral cortex development. Neuropsychologia 28, 517–527. doi:0028-3932(90)90031-I [pii]

Huttenlocher, P.R., 1979a. Synaptic density in human frontal cortex \textemdash {D}evelopmental changes and effects of aging. Brain Res. 163, 195–205.

Huttenlocher, P.R., 1979b. Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res. 163, 195–205. doi:10.1016/0006-8993(79)90349-4

Huttenlocher, P.R., 1978. Synaptic Density in Human Cerebral-Cortex - Developmental-Changes and Effects of Aging. Neurology 28, 375.

Huttenlocher, P.R., Dabholkar, A.S., 1997. Regional differences in synaptogenesis in human cerebral cortex. J Comp Neurol 387, 167–178. doi:10.1002/(SICI)1096-9861(19971020)387:2<167::AID-CNE1>3.0.CO;2-Z [pii]

Huttenlocher, P.R., de Courten, C., Garey, L.J., Van der Loos, H., 1982. Synaptogenesis in human visual cortex--evidence for synapse elimination during normal development. Neurosci Lett 33, 247–252.

Iber, C., Ancoli-Israel, S., Chesson, A.L., Quan, S.F., 2007. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specification, 1st ed. Westchester, IL: American Academy of Sleep Medicine, Westchester, Illinois.

ILAE, 1989. Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia 30, 389–399.

Issa, E.B., Wang, X., 2008. Sensory responses during sleep in primate primary and secondary auditory cortex. J. Neurosci. 28, 14467–80. doi:10.1523/JNEUROSCI.3086-08.2008

Jenni, O.G., Achermann, P., Carskadon, M.A., 2005. Homeostatic sleep regulation in adolescents. Sleep 28, 1446–1454.

Jenni, O.G., Borbély, A.A., Achermann, P., 2004. Development of the nocturnal sleep

electroencephalogram in human infants. Am J Physiol Regul Integr Comp Physiol 286, R528–38. doi:10.1152/ajpregu.00503.200300503.2003 [pii]

Jenni, O.G., Deboer, T., Achermann, P., 2006. Development of the 24-h rest-activity pattern in human infants. Infant Behav Dev 29, 143–152. doi:S0163-6383(05)00097-4 [pii]10.1016/j.infbeh.2005.11.001

Jin, X., Shearman, L.P., Weaver, D.R., Zylka, M.J., De Vries, G.J., Reppert, S.M., 1999. A Molecular Mechanism Regulating Rhythmic Output from the Suprachiasmatic Circadian Clock. Cell 96, 57–68. doi:10.1016/S0092-8674(00)80959-9

Johnson, M.H., 2001. Functional brain development in humans. Nat. Rev. Neurosci. 2, 475–483. doi:10.1038/3508150935081509 [pii]

Jung, T.P., Makeig, S., Humphries, C., Lee, T.W., McKeown, M.J., Iragui, V., Sejnowski, T.J., 2000. Removing electroencephalographic artifacts by blind source separation. Psychophysiology 37, 163–178.

Karni, A., Meyer, G., Rey-Hipolito, C., Jezzard, P., Adams, M.M., Turner, R., Ungerleider, L.G., 1998. The acquisition of skilled motor performance: fast and slow experience-driven changes in primary motor cortex. Proc Natl Acad Sci U S A 95, 861–868.

Keppel, G., 1991. Design and analysis: A researcher's handbook, 3rd ed.

Kirkwood, A., Rioult, M.C., Bear, M.F., 1996. Experience-dependent modification of synaptic plasticity in visual cortex. Nature 381, 526–8. doi:10.1038/381526a0

Kourtis, N., Tavernarakis, N., 2011. Cellular stress response pathways and ageing: intricate molecular relationships. EMBO J. 30, 2520–31. doi:10.1038/emboj.2011.162

Kramer, U., Sue, W.C., Mikati, M.A., 1997. Hypsarrhythmia: frequency of variant patterns and correlation with etiology and outcome. Neurology 48, 197–203.

Kültz, D., 2005. MOLECULAR AND EVOLUTIONARY BASIS OF THE CELLULAR STRESS RESPONSE. Annu. Rev. Physiol. 67, 225–257. doi:10.1146/annurev.physiol.67.040403.103635

Kurth, S., Jenni, O.G., Riedner, B.A., Tononi, G., Carskadon, M.A., Huber, R., 2010a. Characteristics of Sleep Slow Waves in Children and Adolescents. Sleep 33, 475–480.

Kurth, S., Ringli, M., Geiger, A., LeBourgeois, M., Jenni, O.G., Huber, R., 2010b. Mapping of cortical activity in the first two decades of life: a high-density sleep electroencephalogram study. J. Neurosci. 30, 13211–13219. doi:30/40/13211 [pii]10.1523/JNEUROSCI.2532-10.2010

Kurth, S., Ringli, M., Lebourgeois, M.K., Geiger, A., Buchmann, A., Jenni, O.G., Huber, R., 2012. Mapping the electrophysiological marker of sleep depth reveals skill maturation in children and adolescents. Neuroimage 63, 959–65. doi:10.1016/j.neuroimage.2012.03.053

Landau, W.M., Kleffner, PhD, F.R., 1998. Syndrome of Acquired Aphasia with Convulsive Disorder in Children. Neurology 51, 1241–1241. doi:10.1212/WNL.51.5.1241-a

Landolt, H.P., Glatzel, M., Blattler, T., Achermann, P., Roth, C., Mathis, J., Weis, J., Tobler, I., Aguzzi, A., Bassetti, C.L., 2006. Sleep-wake disturbances in sporadic Creutzfeldt-Jakob disease. Neurology 66, 1418–1424. doi:66/9/1418 [pii]10.1212/01.wnl.0000210445.16135.56

Landsness, E.C., Crupi, D., Hulse, B.K., Peterson, M.J., Huber, R., Ansari, H., Coen, M., Cirelli, C., Benca, R.M., Ghilardi, M.F., Tononi, G., 2009. Sleep-dependent improvement in visuomotor learning: a causal role for slow waves. Sleep 32, 1273–1284.

Lante, F., Toledo-Salas, J.C., Ondrejcak, T., Rowan, M.J., Ulrich, D., 2011. Removal of synaptic Ca(2)+-permeable AMPA receptors during sleep. J Neurosci 31, 3953–3961. doi:31/11/3953

[pii]10.1523/JNEUROSCI.3210-10.2011

Larkin, J.E., Yokogawa, T., Heller, H.C., Franken, P., Ruby, N.F., 2004. Homeostatic regulation of sleep in arrhythmic Siberian hamsters. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287, R104–11. doi:10.1152/ajpregu.00676.2003

Lee, G.M.H., Fattinger, S., Mouthon, A.-L., Noirhomme, Q., Huber, R., 2013. Electroencephalogram approximate entropy influenced by both age and sleep. Front. Neuroinform. 7, 33. doi:10.3389/fninf.2013.00033

Léger, J.-F., Stern, E.A., Aertsen, A., Heck, D., 2005. Synaptic integration in rat frontal cortex shaped by network activity. J. Neurophysiol. 93, 281–93. doi:10.1152/jn.00067.2003

Li, X., Cui, S., Voss, L.J., 2008. Using Permutation Entropy to Measure the Electroencephalographic Effects of Sevoflurane. Anesthesiology 109. doi:10.1097/ALN.0b013e318182a91b

Lichtman, J.W., Colman, H., 2000. Synapse Elimination and Indelible Memory. Neuron 25, 269–278. doi:10.1016/S0896-6273(00)80893-4

Liepert, J., Terborg, C., Weiller, C., 1999. Motor plasticity induced by synchronized thumb and foot movements. Exp. Brain Res. 125, 435–439. doi:10.1007/s002210050700

Lim, J., Dinges, D.F., 2008. Sleep deprivation and vigilant attention. Ann. N. Y. Acad. Sci. 1129, 305–22. doi:10.1196/annals.1417.002

Liston, C., Gan, W.B., 2011. Glucocorticoids are critical regulators of dendritic spine development and plasticity in vivo. Proc Natl Acad Sci U S A 108, 16074–16079. doi:1110444108 [pii]10.1073/pnas.1110444108

Liu, Z.-W.W., Faraguna, U., Cirelli, C., Tononi, G., Gao, X.-B.B., 2010. Direct evidence for wake-related increases and sleep-related decreases in synaptic strength in rodent cortex. J. Neurosci. 30, 8671–5. doi:30/25/8671 [pii]10.1523/JNEUROSCI.1409-10.2010

Louis, J., Cannard, C., Bastuji, H., Challamel, M.J., 1997. Sleep ontogenesis revisited: a longitudinal 24-hour home polygraphic study on 15 normal infants during the first two years of life. Sleep 20, 323–333.

Lubenov, E. V., Siapas, A.G., 2008. Decoupling through Synchrony in Neuronal Circuits with Propagation Delays. Neuron 58, 118–131. doi:10.1016/j.neuron.2008.01.036

Luna, B., Garver, K.E., Urban, T.A., Lazar, N.A., Sweeney, J.A., 2004. Maturation of cognitive processes from late childhood to adulthood. Child Dev 75, 1357–1372. doi:10.1111/j.1467-8624.2004.00745.xCDEV745 [pii]

Luna, B., Sweeney, J.A., 2004. The emergence of collaborative brain function: FMRI studies of the development of response inhibition. Ann. N. Y. Acad. Sci. 1021, 296–309. doi:10.1196/annals.1308.0351021/1/296 [pii]

Lustenberger, C., Boyle, M.R., Alagapan, S., Mellin, J.M., Vaughn, B. V, Fröhlich, F., 2016. Feedback-Controlled Transcranial Alternating Current Stimulation Reveals a Functional Role of Sleep Spindles in Motor Memory Consolidation. Curr. Biol. 26, 2127–36. doi:10.1016/j.cub.2016.06.044

Lustenberger, C., Huber, R., 2012. High density electroencephalography in sleep research: potential, problems, future perspective. Front. Neurol. 3, 77. doi:10.3389/fneur.2012.00077

Lux, A.L., Edwards, S.W., Hancock, E., Johnson, A.L., Kennedy, C.R., Newton, R.W., O'Callaghan, F.J., Verity, C.M., Osborne, J.P., 2004. The United Kingdom Infantile Spasms Study comparing vigabatrin with prednisolone or tetracosactide at 14 days: a multicentre, randomised controlled trial. Lancet 364, 1773–1778. doi:S014067360417400X [pii]10.1016/S0140-6736(04)17400-X

Maekawa, K., Ohta, H., Tamai, I., 1980. Transient brain shrinkage in infantile spasms after ACTH treatment. Report of two cases. Neuropadiatrie 11, 80–84. doi:10.1055/s-2008-1071378

Makeig, S., Jung, T.P., Sejnowski, T.J., 2000. Awareness during drowsiness: dynamics and electrophysiological correlates. Can. J. Exp. Psychol. 54, 266–273.

Maquet, P., 2000. Functional neuroimaging of normal human sleep by positron emission tomography. J Sleep Res 9, 207–231. doi:jsr214 [pii]

Maquet, P., Hirsch, E., Metz-Lutz, M.N., Motte, J., Dive, D., Marescaux, C., Franck, G., 1995. Regional cerebral glucose metabolism in children with deterioration of one or more cognitive functions and continuous spike-and-wave discharges during sleep. Brain 118, 1497–1520. doi:10.1093/brain/118.6.1497

Maret, S., Faraguna, U., Nelson, A.B., Cirelli, C., Tononi, G., 2011. Sleep and waking modulate spine turnover in the adolescent mouse cortex. Nat. Neurosci. 14, 1418–20. doi:10.1038/nn.2934

Marshall, L., Helgadottir, H., Molle, M., Born, J., 2006. Boosting slow oscillations during sleep potentiates memory. Nature 444, 610–613. doi:nature05278 [pii]10.1038/nature05278

Massimini, M., Amzica, F., 2001. Extracellular Calcium Fluctuations and Intracellular Potentials in the Cortex During the Slow Sleep Oscillation. J Neurophysiol 85, 1346–1350.

Massimini, M., Ferrarelli, F., Esser, S.K., Riedner, B.A., Huber, R., Murphy, M., Peterson, M.J., Tononi, G., 2007. Triggering sleep slow waves by transcranial magnetic stimulation. Proc Natl Acad Sci U S A 104, 8496–8501. doi:0702495104 [pii]10.1073/pnas.0702495104

Massimini, M., Ferrarelli, F., Huber, R., Esser, S.K., Singh, H., Tononi, G., 2005. Breakdown of cortical effective connectivity during sleep. Science 309, 2228–32. doi:10.1126/science.1117256

Massimini, M., Ferrarelli, F., Murphy, M., Huber, R., Riedner, B., Casarotto, S., Tononi, G., 2010. Cortical reactivity and effective connectivity during REM sleep in humans. Cogn Neurosci 1, 176–183. doi:10.1080/17588921003731578

Massimini, M., Huber, R., Ferrarelli, F., Hill, S., Tononi, G., 2004. The sleep slow oscillation as a traveling wave. J. Neurosci. 24, 6862–6870. doi:10.1523/JNEUROSCI.1318-04.200424/31/6862 [pii]

Massimini, M., Rosanova, M., Mariotti, M., 2003. EEG slow (approximately 1 Hz) waves are associated with nonstationarity of thalamo-cortical sensory processing in the sleeping human. J. Neurophysiol. 89, 1205–13. doi:10.1152/jn.00373.2002

Mensen, A., Riedner, B., Tononi, G., 2016. Optimizing detection and analysis of slow waves in sleep EEG. J. Neurosci. Methods 274, 1–12. doi:10.1016/j.jneumeth.2016.09.006

Moore, R.Y., Eichler, V.B., 1972. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat, Brain Research. doi:10.1016/0006-8993(72)90054-6

Murphy, M., Riedner, B.A., Huber, R., Massimini, M., Ferrarelli, F., Tononi, G., 2009. Source modeling sleep slow waves. Proc Natl Acad Sci U S A 106, 1608–1613. doi:0807933106 [pii]10.1073/pnas.0807933106

Navelet, Y., Benoit, O., Bouard, G., 1982. Nocturnal sleep organization during the first months of life. Electroencephalogr Clin Neurophysiol 54, 71–78.

Ngo, H., Martinetz, T., Born, J., Mölle, M., 2013. Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. Neuron (in press).

Ngo, H.-V. V, Miedema, A., Faude, I., Martinetz, T., Mölle, M., Born, J., 2015. Driving sleep slow oscillations by auditory closed-loop stimulation-a self-limiting process. J. Neurosci. 35, 6630–8.

doi:10.1523/JNEUROSCI.3133-14.2015

Nichols, T.E., Holmes, A.P., 2002. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp 15, 1–25. doi:10.1002/hbm.1058 [pii]

Nickels, K., Wirrell, E., 2008. Electrical status epilepticus in sleep. Semin. Pediatr. Neurol. 15, 50–60. doi:10.1016/j.spen.2008.03.002

Nir, Y., Staba, R.J., Andrillon, T., Vyazovskiy, V. V, Cirelli, C., Fried, I., Tononi, G., 2011. Regional slow waves and spindles in human sleep. Neuron 70, 153–169. doi:S0896-6273(11)00166-8 [pii]10.1016/j.neuron.2011.02.043

Nobili, L., De Gennaro, L., Proserpio, P., Moroni, F., Sarasso, S., Pigorini, A., De Carli, F., Ferrara, M., 2012. Local aspects of sleep: Observations from intracerebral recordings in humans, 1st ed, Progress in Brain Research. Elsevier B.V. doi:10.1016/B978-0-444-59427-3.00013-7

Nobili, L., Ferrara, M., Moroni, F., De Gennaro, L., Russo, G. Lo, Campus, C., Cardinale, F., De Carli, F., 2011. Dissociated wake-like and sleep-like electro-cortical activity during sleep. Neuroimage 58, 612–619. doi:10.1016/j.neuroimage.2011.06.032

O'Callaghan, F.J., Lux, A.L., Darke, K., Edwards, S.W., Hancock, E., Johnson, A.L., Kennedy, C.R., Newton, R.W., Verity, C.M., Osborne, J.P., 2011. The effect of lead time to treatment and of age of onset on developmental outcome at 4 years in infantile spasms: evidence from the United Kingdom Infantile Spasms Study. Epilepsia 52, 1359–1364. doi:10.1111/j.1528-1167.2011.03127.x

Olcese, U., Esser, S.K., Tononi, G., 2010. Sleep and synaptic renormalization: a computational study. J Neurophysiol 104, 3476–3493. doi:jn.00593.2010 [pii]10.1152/jn.00593.2010

Oldfield, R.C., 1971. The assessment and analysis of handedness: The Edinburgh inventory. Neuropsychologia 9, 97–113. doi:10.1016/0028-3932(71)90067-4

Olini, N., Huber, R., 2014. Diurnal changes in electrocorticogram sleep slow-wave activity during development in rats. J. Sleep Res. 23, 261–7. doi:10.1111/jsr.12124

Papadelis, C., Chen, Z., Kourtidou-Papadeli, C., Bamidis, P.D., Chouvarda, I., Bekiaris, E., Maglaveras, N., 2007a. Monitoring sleepiness with on-board electrophysiological recordings for preventing sleep-deprived traffic accidents. Clin. Neurophysiol. 118, 1906–1922.

Papadelis, C., Kourtidou-Papadeli, C., Bamidis, P.D., Maglaveras, N., Pappas, K., 2007b. The effect of hypobaric hypoxia on multichannel {EEG} signal complexity. Clin. Neurophys. 118, 31–52.

Pascual-Leone, A., Manoach, D.S., Birnbaum, R., Goff, D.C., 2002. Motor cortical excitability in schizophrenia. Biol. Psychiatry 52, 24–31. doi:10.1016/S0006-3223(02)01317-3

Paus, T., 2005. Mapping brain maturation and cognitive development during adolescence. Trends Cogn Sci 9, 60–68. doi:S1364-6613(04)00320-1 [pii]10.1016/j.tics.2004.12.008

Paus, T., Collins, D.L., Evans, A.C., Leonard, G., Pike, B., Zijdenbos, A., 2001. Maturation of white matter in the human brain: a review of magnetic resonance studies. Brain Res Bull 54, 255–266. doi:S0361-9230(00)00434-2 [pii]

Perez, E.R., Davidoff, V., Desplartd, P.-A., Deonna, T., 2008. MENTAL AND BEHAVIOURAL DETERIORATION OF CHILDREN WITH EPILEPSY AND CSWS: ACQURIED EPILEPTIC FRONTAL SYNDROME. Dev. Med. Child Neurol. 35, 661–674. doi:10.1111/j.1469-8749.1993.tb11711.x

Perez, M.A., Wise, S.P., Willingham, D.T., Cohen, L.G., 2007. Neurophysiological mechanisms involved in transfer of procedural knowledge. J. Neurosci. 27, 1045–53.

doi:10.1523/JNEUROSCI.4128-06.2007

Petersen, C.C.H., Hahn, T.T.G., Mehta, M., Grinvald, A., Sakmann, B., 2003. Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. Proc. Natl. Acad. Sci. U. S. A. 100, 13638–43. doi:10.1073/pnas.2235811100

Piantoni, G., Poil, S.-S., Linkenkaer-Hansen, K., Verweij, I.M., Ramautar, J.R., Van Someren, E.J.W., Van Der Werf, Y.D., 2013. Individual differences in white matter diffusion affect sleep oscillations. J. Neurosci. 33, 227–33. doi:10.1523/JNEUROSCI.2030-12.2013

Pigorini, A., Sarasso, S., Proserpio, P., Szymanski, C., Arnulfo, G., Casarotto, S., Fecchio, M., Rosanova, M., Mariotti, M., Lo Russo, G., Palva, J.M., Nobili, L., Massimini, M., 2015. Bistability breaks-off deterministic responses to intracortical stimulation during non-REM sleep. Neuroimage 112, 105–13. doi:10.1016/j.neuroimage.2015.02.056

Pincus, S.M., 1994. Greater signal regularity may indicate increased system isolation. Math. Biosci. 122, 161–181. doi:10.1016/0025-5564(94)90056-6

Pincus, S.M., 1991. Approximate entropy as a measure of system complexity. Proc. Natl. Acad. Sci. 88, 2297–2301. doi:10.1073/pnas.88.6.2297

Pincus, S.M., Goldberger, A.L., 1994. Physiological time-series analysis; What does regularity quantify? Am. J. Physiol. 266, H1643–H1656.

Portas, C.M., Krakow, K., Allen, P., Josephs, O., Armony, J.L., Frith, C.D., 2000. Auditory Processing across the Sleep-Wake Cycle. Neuron 28, 991–999. doi:10.1016/S0896-6273(00)00169-0

Rechtschaffen, A., 1998. Current Perspectives on the Function of Sleep. Perspect. Biol. Med. 41, 359–390. doi:10.1353/pbm.1998.0051

Rechtschaffen, A., 1971. The control of sleep, Human behaviour and its control . MA: Shenkman, Cambridge.

Rechtschaffen, A., Kales, A., 1968. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washingt. DC US Public Heal. Serv. US Gov. Print. Off.

Reppert, S.M., Weaver, D.R., 2002. Coordination of circadian timing in mammals. Nature 418, 935–941. doi:10.1038/nature00965

Rezek, I.A., Roberts, S.J., 1998. Stochastic Complexity Measures for Physiological Signal Analysis. IEEE Trans. Biomed. Eng. 45, 1186–1191.

Riedner, B.A., Hulse, B.K., Murphy, M.J., Ferrarelli, F., Tononi, G., 2011. Temporal dynamics of cortical sources underlying spontaneous and peripherally evoked slow waves. Prog. Brain Res. 193, 201–18. doi:10.1016/B978-0-444-53839-0.00013-2

Riedner, B.A., Vyazovskiy, V. V, Huber, R., Massimini, M., Esser, S., Murphy, M., Tononi, G., 2007. Sleep homeostasis and cortical synchronization: III. A high-density EEG study of sleep slow waves in humans. Sleep 30, 1643–1657.

Ringli, M., Huber, R., 2011. Developmental aspects of sleep slow waves: linking sleep, brain maturation and behavior. Prog Brain Res 193, 63–82. doi:B978-0-444-53839-0.00005-3 [pii]10.1016/B978-0-444-53839-0.00005-3

Ringli, M., Souissi, S., Kurth, S., Brandeis, D., Jenni, O.G., Huber, R., 2013. Topography of sleep slow wave activity in children with attention-deficit/hyperactivity disorder. Cortex 49, 340–347. doi:S0010-9452(12)00239-0 [pii]10.1016/j.cortex.2012.07.007

Rioult-Pedotti, M.-S., 2000. Learning-Induced LTP in Neocortex. Science (80-.). 290, 533–536.

doi:10.1126/science.290.5491.533

Rioult-Pedotti, M.S., Friedman, D., Hess, G., Donoghue, J.P., 1998. Strengthening of horizontal cortical connections following skill learning. Nat Neurosci 1, 230–234.

Rodriguez, A. V., Funk, C.M., Vyazovskiy, V. V., Nir, Y., Tononi, G., Cirelli, C., 2016. Why Does Sleep Slow-Wave Activity Increase After Extended Wake? Assessing the Effects of Increased Cortical Firing During Wake and Sleep. J. Neurosci. 36, 12436–12447. doi:10.1523/JNEUROSCI.1614-16.2016

Rosenkranz, K., Kacar, A., Rothwell, J.C., 2007a. Differential modulation of motor cortical plasticity and excitability in early and late phases of human motor learning. J Neurosci 27, 12058–12066. doi:10.1523/JNEUROSCI.2663-07.2007

Rosenkranz, K., Williamon, A., Rothwell, J.C., 2007b. Motorcortical excitability and synaptic plasticity is enhanced in professional musicians. J. Neurosci. 27, 5200–5206. doi:10.1523/Jneurosci.0836-07.2007

Rosenwasser, A.M., Turek, F.W., 2015. Neurobiology of circadian rhythm regulation. Sleep Med. Clin. 10, 403–412. doi:10.1016/j.jsmc.2015.08.003

Rossini, P.M., Barker, A.T., Berardelli, A., Caramia, M.D., Caruso, G., Cracco, R.Q., Dimitrijević, M.R., Hallett, M., Katayama, Y., Lücking, C.H., Maertens de Noordhout, A.L., Marsden, C.D., Murray, N.M.F., Rothwell, J.C., Swash, M., Tomberg, C., 1994. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr. Clin. Neurophysiol. 91, 79–92. doi:10.1016/0013-4694(94)90029-9

Ruddy, K.L., Rudolf, A.K., Kalkman, B., King, M., Daffertshofer, A., Carroll, T.J., Carson, R.G., 2016. Neural Adaptations Associated with Interlimb Transfer in a Ballistic Wrist Flexion Task. Front. Hum. Neurosci. 10, 204. doi:10.3389/fnhum.2016.00204

Sachdev, R.N.S., Ebner, F.F., Wilson, C.J., 2004. Effect of Subthreshold Up and Down States on the Whisker-Evoked Response in Somatosensory Cortex. J. Neurophysiol. 92.

Salzarulo, P., Fagioli, I., 1992. Post-natal development of sleep organization in man: speculations on the emergence of the “S process.” Neurophysiol Clin 22, 107–115.

Sánchez Fernández, I., Takeoka, M., Tas, E., Peters, J.M., Prabhu, S.P., Stannard, K.M., Gregas, M., Eksioglu, Y., Rotenberg, A., Riviello, J.J., Kothare, S. V, Loddenkemper, T., 2012. Early thalamic lesions in patients with sleep-potentiated epileptiform activity. Neurology 78, 1721–7. doi:10.1212/WNL.0b013e3182582ff8

Sanchez-Vives, M. V, McCormick, D.A., 2000. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat. Neurosci. 3, 1027–1034. doi:10.1038/79848

Santostasi, G., Malkani, R., Riedner, B., Bellesi, M., Tononi, G., Paller, K.A., Zee, P.C., 2016. Phase-locked loop for precisely timed acoustic stimulation during sleep. J. Neurosci. Methods 259, 101–14. doi:10.1016/j.jneumeth.2015.11.007

Sayk, F., Teckentrup, C., Becker, C., Heutling, D., Wellhöner, P., Lehnert, H., Dodt, C., 2010. Effects of selective slow-wave sleep deprivation on nocturnal blood pressure dipping and daytime blood pressure regulation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 298, R191–7. doi:10.1152/ajpregu.00368.2009

Scheltens-de Boer, M., 2009. Guidelines for EEG in encephalopathy related to ESES/CSWS in children. Epilepsia 50 Suppl 7, 13–7. doi:10.1111/j.1528-1167.2009.02211.x

Shaw, P., Kabani, N.J., Lerch, J.P., Eckstrand, K., Lenroot, R., Gogtay, N., Greenstein, D., Clasen, L.,

Evans, A., Rapoport, J.L., Giedd, J.N., Wise, S.P., 2008. Neurodevelopmental trajectories of the human cerebral cortex. J. Neurosci. 28, 3586–3594. doi:28/14/3586 [pii]10.1523/JNEUROSCI.5309-07.2008

Shmuelof, L., Krakauer, J.W., Mazzoni, P., 2012. How is a motor skill learned? Change and invariance at the levels of task success and trajectory control. J. Neurophysiol. 108, 578–94. doi:10.1152/jn.00856.2011

Smith, M.C., Hoeppner, T.J., 2003. Epileptic encephalopathy of late childhood: Landau-Kleffner syndrome and the syndrome of continuous spikes and waves during slow-wave sleep. J. Clin. Neurophysiol. 20, 462–72. doi:00004691-200311000-00008 [pii]

Spoormaker, V.I., Schröter, M.S., Gleiser, P.M., Andrade, K.C., Dresler, M., Wehrle, R., Sämann, P.G., Czisch, M., 2010. Development of a Large-Scale Functional Brain Network during Human Non-Rapid Eye Movement Sleep. J. Neurosci. 30, 11379–11387. doi:10.1523/JNEUROSCI.2015-10.2010

Stefan, K., Wycislo, M., Gentner, R., Schramm, A., Naumann, M., Reiners, K., Classen, J., 2006. Temporary occlusion of associative motor cortical plasticity by prior dynamic motor training. Cereb. Cortex 16, 376–385. doi:10.1093/cercor/bhi116

Steriade, M., 2003. The corticothalamic system in sleep. Front. Biosci. 8, d878–99.

Steriade, M., Amzica, F., 1994. Dynamic coupling among neocortical neurons during evoked and spontaneous spike-wave seizure activity. J Neurophysiol 72, 2051–2069.

Steriade, M., Contreras, D., Curro Dossi, R., Nunez, A., 1993a. The slow (< 1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. J Neurosci 13, 3284–3299.

Steriade, M., Nunez, A., Amzica, F., 1993b. A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. J. Neurosci. 13, 3252–3265.

Steriade, M., Nunez, A., Amzica, F., 1993c. Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J Neurosci 13, 3266–3283.

Steriade, M., Timofeev, I., Grenier, F., 2001. Natural waking and sleep states: a view from inside neocortical neurons. J Neurophysiol 85, 1969–1985.

Sterman, M.B., Harper, R.M., Havens, B., Hoppenbrouwers, T., McGinty, D.J., Hodgman, J.E., 1977. Quantitative analysis of infant EEG development during quiet sleep. Electroencephalogr Clin Neurophysiol 43, 371–385.

Stickgold, R., Malia, A., Fosse, R., Propper, R., Hobson, J.A., 2001. Brain-Mind State: I. {Longitudinal} Field Study of Sleep/Wake Factors Influencing Mentation Report Length. Sleep 24, 171–179.

Strijkstra, A.M., Beersma, D.G., Drayer, B., Halbesma, N., Daan, S., 2003. Subjective sleepiness correlates negatively with global alpha (8-12 Hz) and positively with central frontal theta (4-8 Hz) frequencies in the human resting awake electroencephalogram. Neurosci. Lett. 340, 17–20. doi:S0304394003000338 [pii]

Tarokh, L., Carskadon, M.A., 2010. Developmental changes in the human sleep EEG during early adolescence. Sleep 33, 801–809.

Tasali, E., Leproult, R., Ehrmann, D.A., Van Cauter, E., 2008. Slow-wave sleep and the risk of type 2 diabetes in humans. Proc. Natl. Acad. Sci. U. S. A. 105, 1044–9. doi:10.1073/pnas.0706446105

Tassinari, C.A., Rubboli, G., 2006. Cognition and paroxysmal EEG activities: from a single spike to

electrical status epilepticus during sleep. Epilepsia 47 Suppl 2, 40–3. doi:10.1111/j.1528-1167.2006.00686.x

Tassinari, C.A., Rubboli, G., Volpi, L., Billard, C., Bureau, M., 2005. Electrical status epilepticus during slow sleep (ESES or CSWS) including acquired epileptic aplasia(Landau-Kleffner sindrome), in: Roger, J., Bureau, M., Dravet, C., Genton, P., Tassinari, C.A., Wolf, P. (Eds.), Epileptic Syndromes in Infancy, Childhood and Adolescence. John Libbey Eurotext, Montrouge, pp. 295–314.

Tata, D.A., Anderson, B.J., 2010. The effects of chronic glucocorticoid exposure on dendritic length, synapse numbers and glial volume in animal models: implications for hippocampal volume reductions in depression. Physiol Behav 99, 186–193. doi:S0031-9384(09)00307-2 [pii]10.1016/j.physbeh.2009.09.008

te Lindert, B.H.. W., van Someren, E.J.W., 2013. Sleep estimates using microelectromechanical systems (MEMS). Sleep 36, 781–9. doi:10.5665/sleep.2648

Teller, D.Y., 1981. The Development of Visual-Acuity in Human and Monkey Infants. Trends Neurosci. 4, 21–24.

Tesler, N., Gerstenberg, M., Franscini, M., Jenni, O.G., Walitza, S., Huber, R., 2016. Increased frontal sleep slow wave activity in adolescents with major depression. NeuroImage. Clin. 10, 250–6. doi:10.1016/j.nicl.2015.10.014

Thimm, J., Mechler, A., Lin, H., Rhee, S., Lal, R., 2005. Calcium-dependent open/closed conformations and interfacial energy maps of reconstituted hemichannels. J. Biol. Chem. 280, 10646–54. doi:10.1074/jbc.M412749200

Timofeev, I., Bazhenov, M., Seigneur, J., Sejnowski, T., 2012. Neuronal Synchronization and Thalamocortical Rhythms in Sleep, Wake and Epilepsy. doi:NBK98144 [bookaccession]

Timofeev, I., Grenier, F., Bazhenov, M., Sejnowski, T.J., Steriade, M., 2000. Origin of slow cortical oscillations in deafferented cortical slabs. Cereb Cortex 10, 1185–1199. doi:10.1093/cercor/10.12.1185

Timofeev, I., Grenier, F., Steriade, M., 2001. Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. Proc Natl Acad Sci U S A 98, 1924–1929. doi:10.1073/pnas.041430398041430398 [pii]

Tobler, I., Borbely, A.A., 1986. Sleep EEG in the rat as a function of prior waking. Electroencephalogr Clin Neurophysiol 64, 74–76.

Todorov, E., Jordan, M.I., 2002. Optimal feedback control as a theory of motor coordination. Nat. Neurosci. 5, 1226–35. doi:10.1038/nn963

Tononi, G., 2012. Integrated information theory of consciousness: an updated account. Arch. Ital. Biol. 150, 293–329.

Tononi, G., 2008. Consciousness as Integrated Information: a Provisional Manifesto. Biol. Bull. 215, 216–242.

Tononi, G., 2004. An information integration theory of consciousness. BMC Neurosci. 5.

Tononi, G., Cirelli, C., 2014. Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. Neuron 81, 12–34. doi:S0896-6273(13)01186-0 [pii]10.1016/j.neuron.2013.12.025

Tononi, G., Cirelli, C., 2006. Sleep function and synaptic homeostasis. Sleep Med Rev 10, 49–62. doi:S1087-0792(05)00042-0 [pii]10.1016/j.smrv.2005.05.002

Tononi, G., Cirelli, C., 2003. Sleep and synaptic homeostasis: a hypothesis. Brain Res Bull 62, 143–150.

Tononi, G., Massimini, M., 2008. Why Does Consciousness Fade in Early Sleep? Ann. N.Y. Acad. Sci. 1129, 330–334. doi:10.1196/annals.1417.024

Tononi, G., Riedner, B.A., Hulse, B.K., Ferrarelli, F., Sarasso, S., 2010. Enhancing sleep slow waves with natural stimuli. Medicamundi 54, 82–88.

Tononi, G., Sporns, O., 2003. Measuring information integration. BMC Neurosci. 4.

Torsvall, L., Akerstedt, T., 1987. Sleepiness on the job: continuously measured EEG changes in train drivers. Electroencephalogr. Clin. Neurophysiol. 66, 502–511.

Trachsel, L., Edgar, D.M., Seidel, W.F., Craig Heller, H., 1992. Sleep homeostasis in suprachiasmatic nuclei-lesioned rats: effects of sleep deprivation and triazolam administration. Brain Res. 589, 253–261. doi:10.1016/0006-8993(92)91284-L

Turrigiano, G.G., 2008. The self-tuning neuron: synaptic scaling of excitatory synapses. Cell 135, 422–35. doi:10.1016/j.cell.2008.10.008

Uehara, T., Yamasaki, T., Okamoto, T., Koike, T., Kan, S., Miyauchi, S., Kira, J.I., Tobimatsu, S., 2013. Efficiency of a Small-World" Brain Network Depends on Consciousness Level: A Resting-State {fMRI} Study. Cereb. Cortex.

Urbain, C., Di Vincenzo, T., Peigneux, P., Van Bogaert, P., 2011. Is sleep-related consolidation impaired in focal idiopathic epilepsies of childhood? A pilot study. Epilepsy Behav. 22, 380–4. doi:10.1016/j.yebeh.2011.07.023

Van Cauter, E., 2000. Age-Related Changes in Slow Wave Sleep and REM Sleep and Relationship With Growth Hormone and Cortisol Levels in Healthy Men. JAMA 284, 861. doi:10.1001/jama.284.7.861

Van Dongen, H.P.A., Maislin, G., Mullington, J.M., Dinges, D.F., 2003. The cumulative cost of additional wakefulness: Dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction deprivation (vol 26, pg 117, 2003). Sleep 26, 117–126.

Veggiotti, P., Beccaria, F., Guerrini, R., Capovilla, G., Lanzi, G., 1999. Continuous Spike-and-Wave Activity During Slow-Wave Sleep: Syndrome or EEG Pattern? Epilepsia 40, 1593–1601. doi:10.1111/j.1528-1157.1999.tb02045.x

Volgushev, M., 2006. Precise Long-Range Synchronization of Activity and Silence in Neocortical Neurons during Slow-Wave Sleep. J. Neurosci. 26, 5665–5672. doi:10.1523/JNEUROSCI.0279-06.2006

Vyazovskiy, V. V, Achermann, P., Tobler, I., 2007a. Sleep homeostasis in the rat in the light and dark period. Brain Res Bull 74, 37–44.

Vyazovskiy, V. V, Cirelli, C., Pfister-Genskow, M., Faraguna, U., Tononi, G., 2008. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nat Neurosci 11, 200–208. doi:nn2035 [pii]10.1038/nn2035

Vyazovskiy, V. V, Harris, K.D., 2013. Sleep and the single neuron: the role of global slow oscillations in individual cell rest. Nat Rev Neurosci. doi:nrn3494 [pii]10.1038/nrn3494

Vyazovskiy, V. V, Olcese, U., Hanlon, E.C., Nir, Y., Cirelli, C., Tononi, G., 2011. Local sleep in awake rats. Nature 472, 443–447. doi:nature10009 [pii]10.1038/nature10009

Vyazovskiy, V. V, Olcese, U., Lazimy, Y.M., Faraguna, U., Esser, S.K., Williams, J.C., Cirelli, C., Tononi, G., 2009. Cortical firing and sleep homeostasis. Neuron 63, 865–878. doi:S0896-

6273(09)00637-0 [pii]10.1016/j.neuron.2009.08.024

Vyazovskiy, V. V, Riedner, B.A., Cirelli, C., Tononi, G., 2007b. Sleep homeostasis and cortical synchronization: II. A local field potential study of sleep slow waves in the rat. Sleep 30, 1631–1642.

Vyazovskiy, V., Olcese, U., Cirelli, C., Tononi, G., 2012. Prolonged wakefulness alters neuronal responsiveness to local electrical stimulation of the neocortex in awake rats. J. Sleep Res. 22, 239–250.

Walsh, J.K., Perils, M., Rosenthal, M., Krystal, A., Jiang, J., Roth, T., 2006. Tiagabine increases slow-wave sleep in a dose-dependent fashion without affecting traditional efficacy measures in adults with primary insomnia. J. Clin. Sleep Med. 2, 35–41.

Walsh, J.K., Snyder, E., Hall, J., Randazzo, A.C., Griffin, K., Groeger, J., Eisenstein, R., Feren, S.D., Dickey, P., Schweitzer, P.K., 2008. Slow wave sleep enhancement with gaboxadol reduces daytime sleepiness during sleep restriction. Sleep 31, 659–672.

Walter, P., Ron, D., 2011. The Unfolded Protein Response: From Stress Pathway to Homeostatic Regulation. Science (80-.). 334.

Watanabe, K., Negoro, T., Aso, K., Matsumoto, A., 1993. Reappraisal of interictal electroencephalograms in infantile spasms. Epilepsia 34, 679–685.

Wellman, C.L., 2001. Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. J Neurobiol 49, 245–253. doi:10.1002/neu.1079 [pii]

Werth, E., Dijk, D.J., Achermann, P., Borbely, A.A., 1996. Dynamics of the sleep EEG after an early evening nap: experimental data and simulations. Am J Physiol 271, R501–10.

Whitford, T.J., Rennie, C.J., Grieve, S.M., Clark, C.R., Gordon, E., Williams, L.M., 2007. Brain maturation in adolescence: concurrent changes in neuroanatomy and neurophysiology. Hum Brain Mapp 28, 228–237.

Wilhelm, I., Wagner, U., Born, J., 2011. Opposite effects of cortisol on consolidation of temporal sequence memory during waking and sleep. J. Cogn. Neurosci. 23, 3703–12. doi:10.1162/jocn_a_00093

Xie, L., Kang, H., Xu, Q., Chen, M.J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D.J., Nicholson, C., Iliff, J.J., Takano, T., Deane, R., Nedergaard, M., 2013. Sleep Drives Metabolite Clearance from the Adult Brain. Science (80-.). 342.

Yang, G., Gan, W.-B., 2012. Sleep contributes to dendritic spine formation and elimination in the developing mouse somatosensory cortex. Dev. Neurobiol. 72, 1391–1398. doi:10.1002/dneu.20996

Zhang, X., de Beukelaar, T.T., Possel, J., Olaerts, M., Swinnen, S.P., Woolley, D.G., Wenderoth, N., 2011. Movement Observation Improves Early Consolidation of Motor Memory. J. Neurosci. 31, 11515–11520. doi:10.1523/JNEUROSCI.6759-10.2011

Zhang, X.-S., Roy, R.J., Jensen, E.W., 2001. {EEG} Complexity as a Measure of Depth of Anesthesia for Patients. IEEE Trans. Biomed. Eng. 48, 1424–1433.

Zhou, X., Ferguson, S.A., Matthews, R.W., Sargent, C., Darwent, D., Kennaway, D.J., Roach, G.D., 2011. Dynamics of neurobehavioral performance variability under forced desynchrony: evidence of state instability. Sleep 34, 57–63.

Zuo, Y., Lin, A., Chang, P., Gan, W.B., 2005. Development of long-term dendritic spine stability in diverse regions of cerebral cortex. Neuron 46, 181–189.

7. Curriculum Vitae

Name	Sara Corinna Fattinger
Date of Birth	June 23th 1986 in Zurich, Switzerland
Place of Origin	Arlesheim (BL), Switzerland

Education

12/2006	Maturität, main focus: economy and law, high school Laufen (Switzerland)
09/2007 - 12/2011	Studies in Human movement sciences, ETH Zurich (Switzerland)
since 09/2012	Studies in Human medicine, University of Zurich (Switzerland)
10/2012 - 12/2016	International Ph.D. program in neuroscience of the ETH Zurich and the University of Zurich (Switzerland)

Academic degrees

09/2010	Bachelor of science in human movement sciences of the ETH Zurich (Switzerland)
12/2011	Master of science in human movement sciences of the ETH Zurich (Switzerland)
09/2015	Bachelor of Medicine of the University Zurich (Switzerland)

Professional experiences

08/2007 – 09/2007	Internship at *Kunde & Co,* Copenhagen (Denmark)
09/2010 – 12/2010	Tutorial assistant at the Institute of Physiology, University of Zurich (Switzerland)
10/2010 – 12/2010	Internship in the Neuropsychology Institute, University of Zurich (Switzerland)

8. Publication list

Peer-reviewed journal articles

First authorship

Sara Fattinger, Oskar G. Jenni, Bernhard Schmitt, Peter Achermann, Reto Huber (2013). Overnight changes in the slope of sleep slow waves during infancy. *Sleep* 37(2): 245-253

Sara Fattinger, Bernhard Schmitt, Bigna K. Bölsterli Heinzle, Hanne Critelli, Oskar G. Jenni, Reto Huber (2015). Impaired slow wave sleep downscaling in patients with infantile spasms. *European Journal of Paediatric Neurology* 19: 134-142

Sara Fattinger[*], Toon T. de Beukelaar[*], Kathy L. Ruddy[*], Carina Volk, Joshua A. Herbst, Richard H. R. Hahnloser, Nicole Wenderoth[**], Reto Huber[**]. Deep sleep maintains learning efficiency of the human brain. Resubmitted to *Nature Communications*

* Equal contribution

** Equal contribution

Sara Fattinger, Salome Kurth, Maya Ringli, Oskar G. Jenni, Reto Huber. Oscillatory theta events in the waking electroencephalogram of children resemble local aspects of sleep during wakefulness. Submitted

Co- authorship

Gerick M. H. Lee, **Sara Fattinger**, Anne-Laure Mouthon, Quentin Noirhomme, Reto Huber (2013). Electroencephalogram approximate entropy influenced by both age and sleep. *Frontiers in Neuroinformatics* 7:33

Bigna K. Bölsterli Heinzle**, Fattinger Sara**, Salome Kurth, Monique K. Lebourgeois, Maya Ringli, Thomas Bast, Hanne Critelli, Bernhard Schmitt, Reto Huber (2014). Spike wave location and density disturb sleep slow waves in patients with CSWS (continuous spike waves during sleep). *Epilepsia* 4: 584-591

Abstracts

Oral presentation

Sara Fattinger, Oskar G. Jenni, Bernhard Schmitt, Peter Achermann, Reto Huber (09/2012). Changes of sleep slow wave characteristics during infancy - a longitudinal study. *21th Congress of the European Sleep Research Society, in Paris, France.*

Sara Fattinger, Oskar G. Jenni, Bernhard Schmitt, Peter Achermann, Reto Huber (05/2013). Overnight changes in the slope of sleep slow waves during infancy. *Annual Conference 2013 of the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology (SSSSC) in Aarau, Switzerland.*

Sara Fattinger, Bernhard Schmitt, Bigna K. Bölsterli Heinzle, Hanne Critelli, Oskar G. Jenni, Reto Huber (09/2013). Impaired slow wave sleep downscaling in infantile spasms with hypsarrhythmia. *European Paediatric Neurology Society Congress, in Brussels, Belgium.*

Sara Fattinger, Carina Volk, Toon T. de Beukelaar, Joshua A. Herbst, Nicole Wenderoth, Reto Huber (10/2015). Auditory stimulation time-locked to the down-phase of sleep slow waves in humans. *5th FZK/CRC Retreat in Au, Switzerland.*

Sara Fattinger, Toon T. de Beukelaar, Kathy L. Ruddy, Carina Volk, Natalie Heyse, Joshua A. Herbst, Richard H. R. Hahnloser, Nicole Wenderoth, Reto Huber (09/2016). Closed-loop auditory stimulation time-locked to the down-phase of sleep slow waves provides a tool to locally interfere with brain activity during deep sleep. *23th Congress of the European Sleep Research Society, in Bologna, Italy.*

Poster presentation

Sara Fattinger, Oskar G. Jenni, Bernhard Schmitt, Peter Achermann, Reto Huber (08/2012). Changes of sleep slow wave characteristics during infancy - a longitudinal study. *8th ZIHP Symposium in Zurich, Switzerland*

Sara Fattinger, Salome Kurth, Maya Ringli, Oskar G. Jenni, Reto Huber (09/2012). Characteristics of theta waves during wakefulness – a marker of synaptic strength? *21th Congress of the European Sleep Research Society, in Paris, France.*

Sara Fattinger, Bernhard Schmitt, Bigna K. Bölsterli Heinzle, Hanne Critelli, Oskar G. Jenni, Reto Huber (05/2013). Impaired slow wave sleep downscaling in infantile spasms with hypsarrhythmia. *Gemeinsame Jahrestagung der Deutschen, Österreichischen und Schweizerischen Sektionen der internationalen Liga gegen Epilepsie, in Interlaken, Switzerland*

Sara Fattinger, Bernhard Schmitt, Bigna K. Bölsterli Heinzle, Hanne Critelli, Oskar G. Jenni, Reto Huber (09/2014). Impaired slow wave sleep downscaling in patients with hypsarrhythmia. *22th Congress of the European Sleep Research Society, in Tallinn, Estonia.*

Sara Fattinger, Fatime Bislimi, Carina Volk, Adelinn Chiang, Joshua A. Herbst, Oskar G. Jenni, Reto Huber (06/2015). Local slow wave deprivation during sleep by auditory time-looked stimulation based on real time slow wave detection. *Annual Conference 2015 of the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology (SSSSC) in Interlaken, Switzerland.*

Sara Fattinger, Fatime Bislimi, Carina Volk, Toon T. de Beukelaar, Joshua A. Herbst, Nicole Wenderoth, Reto Huber (09/2015). Closed-loop auditory stimulation time-locked to the down-phase of sleep slow waves in humans. *ZNZ Symposium in Zurich, Switzerland.*

Sara Fattinger, Toon T. de Beukelaar, Carina Volk, Joshua A. Herbst, Richard H. R. Hahnloser, Nicole Wenderoth, Reto Huber (03/2016). Closed-loop auditory stimulation time-locked to the down-phase of sleep slow waves interferes with brain activity during deep sleep. *Gordon Research Conference on Sleep Regulation & Function, Galveston (TX), USA.*

Sara Fattinger, Carina Volk, Fatime Bislimi, Toon T. de Beukelaar, Joshua A. Herbst, Nicole Wenderoth, Reto Huber (06/2016). Closed-loop auditory stimulation time-locked to the down-phase of sleep slow waves interferes with brain activity during deep sleep. *Annual Conference 2016 of the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology (SSSSC) in Basel, Switzerland.*

9. Acknowledgement

First I would like to thank Prof. David P. Wolfer for his interest in my work and for being chair of my thesis committee.

Thank you to my main supervisor and principal investigator of my thesis project, Prof. Reto Huber. I am very grateful and fortunate that he gave me the opportunity for this Ph.D. project in addition to my medical studies. It was a very inspiring and beneficial time, during which I was able to strongly profit from his great scientific experience and knowledge. I greatly appreciate that he provided me with the opportunity to follow my own ideas when investigating and developing the auditory closed-loop system. This work would simply not have been possible without his great support, trust, valuable inputs and encouragements.

I would like to thank Prof. Oskar Jenni for all his support and his invaluable comments on our research articles. I greatly appreciated the opportunity to reanalyse the unique dataset of infant's overnight sleep EEG which he had collected.

Thank you to Prof. Bernhard Schmitt for all his support, valuable clinical inputs and fruitful discussions. I deeply appreciate his interest and openness for clinical translation of basic research. His trust in my abilities gave me the opportunity to combine the field of childhood epilepsy with basic sleep research for which I am very grateful.

Another thank you goes to Bigna Bölsterli Heinzle and Hanne Critelli for the valuable clinical inputs, advices and the tremendous support in handling and analysing pathological EEGs.

Furthermore, I would like to thank the collaborators of the closed-loop system project, Nici Wenderoth, Kathy Ruddy, Toon de Beukelaar, Joshua Herbst and Richard Hahnloser for the opportunity to conduct this study. I am very grateful for the excellent cooperation and their vast expertise, which made the project a success.

Without the contribution and support of many other people this work would not have been possible. I would like to take this opportunity to express my gratitude to all of them:

To Joshua Herbst for developing and programming the closed-loop system algorithm and your effort and patience during this process. I will never forget the afternoon when we were trying to reach Harpreet in India or our first pilot recordings with the closed-loop system. Thank you for your support and our good friendship.

To Harpreet Singh, Peter Achermann and Roland Dürr for your precious assistance and support regarding technical questions.

To the team at the EEG laboratory at Kispi for your continuous support as well as providing and preparing the rooms for those many overnight EEG measurements.

To Carina Volk, Fatime Bislimi, Natalie Heyse, Adelinn Chiang and Manuela Rytz for the numerous nights you spent at the sleep laboratory to help with data acquisition, your support was invaluable.

To Flavia Wehrle for reading and editing this thesis. Also to Tanja Kakebeeke, Maya Ringli and Corina Noventa for their useful comments on the manuscript.

To my wonderful current and former co-workers of the sleep research group at Kispi: Salome Kurth, Maya Ringli, Anne-Laure Mouthon, Caroline Lustenberger, Fiona Pugin, Noemi Tesler, Nadja Olini, Angelina Maric, Gerick Lee, Carina Volk, Ines Wilhelm, Flavia Wehrle, Sabine Groch, Bigna Bölsterli, Fatime Bislimi, Natalie Heyse, Gaetan Petit, Valeria Jaramillo and Georgia Sousouri. A big thank you to all of you and for your never-ending support and understanding. You greatly relieved me of much administrative and organizational work, especially in times when the work load of my medical studies was piling up. I feel incredibly fortunate to maintain such amazing friendships and to be sharing the ups and downs of my Ph.D. adventure with all of you.

To all the participants of the study, who made this research possible in the first place. It was a great pleasure to getting to know you. I greatly appreciate the time, effort and interest you put into this project.

To all my friends, I am beyond grateful for your constant and tireless support. Thank you for your understanding and patience when I was occupied with a great deal of work and thank you foremost for always being there for me - no matter what.

To my parents and my three amazing brothers. Thank you for always believing in me and making all of this even possible. Thank you for giving me the space I needed and for intuitively knowing when to ask about the Ph.D. and the medical studies and when not to. I am deeply grateful for your unconditional support. Thank you for accompanying me on this and many other journeys.

Finally to my boyfriend Lukas. Thank you for always believing in my abilities and the many reassurances at just the right time. You offered me the support and the strong backing I needed during this intense period. Without you I wouldn’t have made it this far. Thank you for always being by my side, for putting your needs and wishes in the back and being so patient with me. Now we have done it.